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(54) **WHEAT HAVING RESISTANCE TO GLYPHOSATE DUE TO ALTERATIONS IN 5-ENOL- PYRUVYL SHIKIMATE-3 PHOSPHATE SYNTHASE**

(71) Applicant: **Arcadia Biosciences, Inc.**, Seattle, WA (US)

(72) Inventors: **Charles Paul Moehs**, Seattle, WA (US); **Michael N. Steine**, Kent, WA (US); **Jessica C. Mullenberg**, Kirkland, WA (US); **Ann J. Slade**, Bellevue, WA (US)

(73) Assignee: **ARCADIA BIOSCIENCES, INC.**, Davis, CA (US)

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(52) **U.S. Cl.**

CPC **A01H 6/4678** (2018.05); **A01H 5/10** (2013.01); **A23L 7/10** (2016.08); **C12N 15/8274** (2013.01); **C12N 15/8275** (2013.01)

(58) **Field of Classification Search**

CPC **C12N 15/8275**; **A01H 5/10**
See application file for complete search history.

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Primary Examiner — Weihua Fan

(74) *Attorney, Agent, or Firm* — Troutman Pepper

Hamilton Sanders LLP (Rochester)

(57) **ABSTRACT**

Plants with resistance to glyphosate are disclosed herein. In one embodiment, the disclosure relate to human induced non-transgenic mutations in the EPSPS gene in plants.

19 Claims, 1 Drawing Sheet

Specification includes a Sequence Listing.

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wheat	..MAMAAAAT	VAASASSSAV	SLDRAAPAHP	RRLRMPAA.R	AAHRGAVRLW
rice	MAATMASNAA	AAAAVSLDQA	VAASAAFSSR	KQLRLPAAAR	GGMPVRVRAR
Consensus	..aaMAaaaAa	aAAaaaSlDaa	saarAAfahR	rrLR\$PAA.R	aahRgaVRar
	51				100
wheat	GPRGAA..AR	ATSVAAPAAP	AGAEVVLQP	IREISGAVQL	PGSKSLSNRI
rice	GRREAVVVAS	ASSSSVAAPA	AKAEEIVLQP	IREISGAVQL	PGSKSLSNRI
Consensus	GrReAa..Ar	AsSsaaaAaa	AgAEE!VLQP	IREISGAVQL	PGSKSLSNRI
	101				150
wheat	LLLSALSEGT	TVVDNLLNSE	DVHYMLEALE	ALGLSVEADK	VAKRAVVVGC
rice	LLLSALSEGT	TVVDNLLNSE	DVHYMLEALK	ALGLSVEADK	VAKRAVVVGC
Consensus	LLLSALSEGT	TVVDNLLNSE	DVHYMLEALe	ALGLSVEADK	VAKRAVVVGC
	151				200
wheat	GGRFPVEKDA	KEEVKLFILGN	AGTAMRPLTA	AVVAAGGNAT	YVLDGVPRMR
rice	GGKFPVEKDA	KEEVQLFLGN	AGTAMRPLTA	AVTAAGGNAT	YVLDGVPRMR
Consensus	GGrFPVEKDA	KEEVqLFLGN	AGTAMRPLTA	AVtAAGGNAT	YVLDGVPRMR
	201				250
wheat	ERPIGDLVVG	LQQLGADVDC	FLGTNCPVVR	INGKGLPFGG	KVKLSGSISS
rice	ERPIGDLVVG	LKQLGADVDC	FLGTECPPVR	VKGIGGLPFGG	KVKLSGSISS
Consensus	ERPIGDLVVG	LqQLGADVDC	FLGT#CPPVR	!nGiGGLPFGG	KVKLSGSISS
	251				300
wheat	QYLSLLMAA	PLALEDVEIE	IIDKLISVPY	VENTLKLMEER	FGVTAEHSDS
rice	QYLSALLMAA	PLALGDVEIE	IIDKLISIPY	VENTLRLMEER	FGVKAEHSDS
Consensus	QYLSaLLMAA	PLALeDVEIE	IIDKLIS!PY	VENTLrLMEER	FGVkaEHSDS
	301				350
wheat	WDRFYIKGGQ	KYKSPGNAYV	EGDASSASYF	LAGAAITGGT	VTVEGCGTTS
rice	WDRFYIKGGQ	KYKSPGNAYV	EGDASSASYF	LAGAAITGGT	VTVQCGTTS
Consensus	WDRFYIKGGQ	KYKSPGNAYV	EGDASSASYF	LAGAAITGGT	VTV#GCGTTS
	351				400
wheat	LQGDVKFAEV	LEMMGAKVTW	TDTSVTVTGP	PRQPFGRKHL	KAVDVMNMNM
rice	LQGDVKFAEV	LEMMGAKVTW	TDTSVTVTGP	PREPYGKKHL	KAVDVMNMNM
Consensus	LQGDVKFAEV	LEMMGAKVTW	TDTSVTVTGP	PR#P%GrKHL	KAVDVMNMNM
	401				450
wheat	PDVAMTLAVV	ALFADGPTAI	RDVASWRVKE	TERMVAIRTE	LTKLGATVEE
rice	PDVAMTLAVV	ALFADGPTAI	RDVASWRVKE	TERMVAIRTE	LTKLGASVEE
Consensus	PDVAMTLAVV	ALFADGPTAI	RDVASWRVKE	TERMVAIRTE	LTKLGAsVEE
	451				500
wheat	GPDYCIITPP	EKLNITAITD	YDDHRMAMAF	SLAACAEVPV	TIRDPGCTRK
rice	GPDYCIITPP	EKLNITAITD	YDDHRMAMAF	SLAACADVPV	TIRDPGCTRK
Consensus	GPDYCIITPP	EKLNITAITD	YDDHRMAMAF	SLAACA#VPV	TIRDPGCTRK
	501	515			
wheat	TFPNYFDVLS	TFVKN			
rice	TFPNYFDVLS	TFVRN			
Consensus	TFPNYFDVLS	TFVrN			

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**WHEAT HAVING RESISTANCE TO
GLYPHOSATE DUE TO ALTERATIONS IN
5-ENOL- PYRUVYLSHIKIMATE-3
PHOSPHATE SYNTHASE**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a continuation patent application of U.S. patent application Ser. No. 15/740,876 filed Dec. 29, 2017, which is a U.S. national phase application under § 371 of PCT 2016/040760 filed Jul. 1, 2016, which claims priority to U.S. Provisional Patent Application No. 62/188,360 filed Jul. 2, 2015, which is incorporated herein by reference in its entirety.

FIELD

This disclosure relates to mutations in one or more 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) homoeologous genes of wheat and wheat plants and plant parts having said mutations in at least one of their EPSPS homoeologous gene sequences. In one embodiment, the mutation is a human induced non-transgenic mutation. In another embodiment, the disclosure relates to wheat having resistance to the herbicide glyphosate as a result of mutations in at least one of their EPSPS homoeologous genes.

SUBMISSION OF SEQUENCE LISTING

The contents of the electronic submission of the text file Sequence Listing, which is named ARC-38879-A-US.txt, which was created Sep. 20, 2019, and is 39 KB in size, is incorporated herein by reference in its entirety.

BACKGROUND

The control of weeds in wheat cultivation would be considerably improved if the wheat could be made resistant to the lethal effects of the herbicide used by commercial growers to control the weeds. One such herbicide is glyphosate, which inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Because EPSPS activity is required for the biosynthesis of a number of essential compounds including amino acids and lignin, its inhibition is lethal to plants. Glyphosate is a very effective herbicide and is widely used in commercial agriculture.

A number of crop plants, including canola, corn, soybeans and cotton, have been transgenically engineered to over-express an altered bacterial EPSPS. The altered bacterial EPSPS does not bind glyphosate but still retains high affinity for the plants' endogenous substrates, including phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid. Fields planted with these genetically engineered crops can be sprayed with glyphosate during the growing season to control weeds since the crops expressing the altered bacterial EPSPS survive.

Because of its tremendous commercial value, transgenic glyphosate resistant wheat has been developed (U.S. Pat. No. 7,071,325) but it has not been released commercially due largely to the lack of acceptance by consumers of genetically modified (GM) foods (Stokstad, *Science* 304: 1088-1089, 2004). Because many consumers have preferences against GM crops, particularly GM food crops, attempts have been made to develop glyphosate resistant wheat through non-transgenic means. For example, the use of recombinogenic oligonucleotides to mutate endogenous

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EPSPS genes has been proposed (US Patent Application 2008/0256668) but it was not demonstrated that glyphosate resistant *Arabidopsis* plants could in fact be regenerated using this methodology and it was not shown that this methodology would work in wheat.

Despite previous investigations into the properties of EPSPS and the conserved nature of the glyphosate binding pocket, there is variation among species in the particular amino acid changes in the EPSPS enzyme that lead to resistance. For example, an alteration of a threonine to isoleucine at position 97 in the *E. coli* EPSPS leads to sensitivity to glyphosate and decreased affinity of the enzyme for its natural substrate phosphoenolpyruvate (PEP) (Funke et al., *Journal of Biological Chemistry* 284: 9854-9860, 2009), while, by contrast, alteration of the equivalent threonine to isoleucine in the maize EPSPS leads to reduced inhibition by glyphosate (U.S. Pat. No. 6,566,587).

In conclusion, there is a continuing commercial interest in the development of glyphosate resistant wheat that is not the product of genetic engineering and would therefore be acceptable to all consumers. Previous work has demonstrated that the transgenic over-expression of a mutated bacterial EPSPS results in glyphosate resistance in a number of crops including wheat. However, to date, there is no evidence that mutations of one or more endogenous EPSPS genes will lead to resistance in any crop plant, particularly in a hexaploid plant such as wheat. Further, the nature of the mutations that could result in glyphosate resistance is not known since species specific differences have been noted despite the fact that glyphosate binding domain is highly conserved across EPSPSs.

To meet the need for non-transgenic glyphosate resistant wheat, we have used a target-selected mutagenesis screening method to create and identify specific alterations in each of the three homoeologous copies of hexaploid wheat EPSPS. Novel partial genomic DNA sequences for the three wheat EPSPS homoeologous genes were determined and then used to identify individual wheat plants that contained specific alterations in the active site region of each EPSPS homoeologue. Using the mutations as selective markers, crosses were made to generate plants that were homozygous for preferred alterations in all of the homoeologous copies of EPSPS. Because these wheat plants are resistant to glyphosate without the inclusion of foreign DNA in their genomes, they will be acceptable to consumers and wheat breeders alike. Such a non-transgenic wheat cultivar with resistance to glyphosate as a result of human-induced non-transgenic mutations in one or more EPSPS homoeologous genes would have tremendous value for wheat based food products such as breads, cakes, cookies, tortillas and crackers.

SUMMARY

In one embodiment, the disclosure relates to plants and plant parts with one or mutations in an EPSPS gene that result in glyphosate resistant plants, including but not limited to barley, wheat and rye plants. In one embodiment, the disclosure relates to plants and plant parts with one or mutations in an EPSPS gene that result in glyphosate tolerant plants, including but not limited to barley, wheat and rye plants.

In one embodiment, the mutations are human-induced non-transgenic mutations. In yet another embodiment, the disclosure relates to plants with modified genes, wherein the genes were modified by genomic editing and contribute to plants with resistance to glyphosate.

In one embodiment, the plants discussed herein include wheat, barley and rye. In one embodiment, the disclosure relates to grains from plants and plant parts with one or mutations in an EPSPS gene.

In one embodiment, the disclosure relates to plants with non-transgenic mutations in one or more EPSPS genes, or homologous genes, which result in plants with resistance to glyphosate. In one embodiment, the disclosure relates to non-transgenic mutations in the EPSPS gene, wherein said mutations result in plants with resistance to glyphosate.

In one embodiment, one or more mutations are in the EPSPS gene of the wheat A genome. In another embodiment, one or more mutations are in the EPSPS gene of the wheat B genome. In another embodiment, one or more mutations are in the EPSPS gene of the D genome.

In one embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In another embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the A genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations and multiple mutations in the EPSPS gene of the B genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In another embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the A genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations and multiple mutations in the EPSPS gene of the D genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In another embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the B genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations and multiple mutations in the EPSPS gene of the D genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In another embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the A genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations and multiple mutations in the EPSPS gene of the B genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations and multiple mutations in the EPSPS gene of the D genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In one embodiment, the disclosure relates to a wheat plant comprising at least two mutations in an EPSPS gene of the A genome, wherein the mutated EPSPS gene of the A genome encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9.

In yet another embodiment, the disclosure relates to a wheat plant comprising two mutations in an EPSPS gene of the D genome, wherein the mutated EPSPS gene of the D genome encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9.

In another embodiment, the disclosure relates to a wheat plant, wheat seeds, wheat plant parts, and progeny thereof with resistance to glyphosate as compared to wild type wheat plant, wheat seeds, wheat plant parts, and progeny thereof.

In another embodiment, the disclosure relates to a wheat plant, wheat seeds, wheat plant parts, and progeny thereof

having resistance to glyphosate as compared to the wild type wheat plant, wherein the resistance to glyphosate is caused by a human-induced non-transgenic mutation in one or more of the wheat plant's EPSPS genes. In another embodiment, the disclosure relates to wheat plants with resistance to glyphosate as compared to the wild type wheat plant and normal germination and viability rates.

In another embodiment, the altered EPSPS protein, which is coded for by the EPSPS gene having a mutation, has altered affinity for glyphosate. In yet another embodiment, the altered EPSPS protein retains substantial affinity for the enzyme's endogenous substrates.

In another embodiment, the disclosure relates to a wheat plant containing one or more mutated EPSPS genes, as well as seeds, pollen, plant parts and progeny of that plant.

In another embodiment, the disclosure relates to food and food products incorporating wheat seeds and wheat flour with an altered EPSPS protein, which has reduced affinity for glyphosate caused by a human-induced non-transgenic mutation in one or more EPSPS genes.

In another embodiment, this disclosure relates to a wheat plant having one or more EPSPS proteins with reduced affinity for glyphosate compared to the wild type wheat plants, created by the steps of obtaining plant material from a parent wheat plant, inducing at least one mutation in at least one copy of a EPSPS gene of the plant material by treating the plant material with a mutagen to create mutagenized plant material (e.g., seeds or pollen), analyzing progeny wheat plants to detect at least one mutation in at least one copy of an EPSPS gene, selecting progeny wheat plants that have at least one mutation in at least one copy of an EPSPS gene, crossing progeny wheat plants that have at least one mutation in at least one copy of an EPSPS gene with other progeny wheat plants that have at least one mutation in a different copy of a EPSPS gene, and repeating the cycle of identifying progeny wheat plants having mutations and crossing the progeny wheat plants having mutations with other progeny wheat plants having mutations to produce progeny wheat plants with an EPSPS protein with reduced affinity for glyphosate. In another embodiment, the method comprises growing or using the mutagenized plant material to produce progeny wheat plants.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 shows the *Oryza sativa* EPSPS mRNA (NCBI Reference Sequence NM_001063247).

SEQ ID NO:2 shows the *Oryza sativa* EPSPS genomic DNA (NCBI Reference Sequence NC_008399).

SEQ ID NO: 3 shows the EPSPS protein encoded by SEQ ID NO: 2 (NCBI Reference Sequence NP_001056712).

SEQ ID NOS: 4-5 show the DNA sequences for *Triticum aestivum* EPSPS-specific primers used for genomic sequencing.

SEQ ID NO: 6 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the A genome of wheat.

SEQ ID NO: 7 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the B genome of wheat.

SEQ ID NO: 8 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the D genome of wheat.

SEQ ID NO: 9 shows the amino acid sequence for the wheat EPSPS protein.

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SEQ ID NOs: 10-13 show the DNA sequences for wheat EPSPS-specific primers used for mutation detection.

SEQ ID NOs: 14-17 show primers useful for mutation detection.

SEQ ID NO. 18 shows the amino acid sequence of the active region of the wheat EPSPS protein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a sequence alignment of the rice EPSPS protein (SEQ ID NO. 20) and the wheat EPSPS protein (SEQ ID NO. 19). There is substantial similarity between the rice and wheat EPSPS proteins (consensus shown as SEQ ID NO. 21).

DETAILED DESCRIPTION

Definitions

The numerical ranges in this disclosure are approximate, and thus may include values outside of the range unless otherwise indicated. Numerical ranges include all values from and including the lower and the upper values, in increments of one unit, provided that there is a separation of at least two units between any lower value and any higher value. As an example, if a compositional, physical or other property, such as, for example, molecular weight, viscosity, etc., is from 100 to 1,000, it is intended that all individual values, such as 100, 101, 102, etc., and sub ranges, such as 100 to 144, 155 to 170, 197 to 200, etc., are expressly enumerated. For ranges containing values which are less than one or containing fractional numbers greater than one (e.g., 1.1, 1.5, etc.), one unit is considered to be 0.0001, 0.001, 0.01 or 0.1, as appropriate. For ranges containing single digit numbers less than ten (e.g., 1 to 5), one unit is typically considered to be 0.1. These are only examples of what is specifically intended, and all possible combinations of numerical values between the lowest value and the highest value enumerated, are to be considered to be expressly stated in this disclosure. Numerical ranges are provided within this disclosure for, among other things, relative amounts of components in a mixture, and various temperature and other parameter ranges recited in the methods.

As used herein, the term "allele" is any of one or more alternative forms of a gene, all of which relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes. In a tetraploid or hexaploid cell or organism, such as wheat, the two alleles of a given gene on one of the genomes occupy corresponding loci on a pair of homologous chromosomes and the two alleles of the same gene occupying the same loci on another of the genomes such as the A or B genomes of tetraploid, or the A, B or D genomes of hexaploid wheat are said to be homoeologous to the gene of the first genome and to be present on homoeologous chromosomes.

As used herein, the terms "altering," "increasing," "increased," "reducing," "reduced," "inhibited" or the like are considered relative terms, i.e. in comparison with the wild-type or unaltered state. The "level of a protein" refers to the amount of a particular protein, for example EPSPS, which may be measured by any means known in the art such as, for example, Western blot analysis or other immunological means.

As used herein, "altered EPSPS protein activity" refers to an EPSPS protein with reduced affinity for glyphosate. In

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one embodiment, the EPSPS protein may have altered affinity for glyphosate but retain substantial affinity for plant or endogenous substrates. It would be appreciated that the level of EPSPS activity might be altered in a mutant but not the expression level (amount) of the protein itself. Conversely, the amount of protein might be altered but the activity remain the same if a more or less active protein is produced. Reductions in both amount and activity are also possible such as, for example, when a gene encoding the protein is inactivated. In certain embodiments, the reduction in the level of protein or reduced affinity for glyphosate is by at least 10% or by at least 20% or by at least 30% or by at least 40% or by at least 50% or by at least 60%, or by at least 70%, or by at least 80% or by at least 85% or by at least 90% or at least 95% as compared to the level of protein or affinity for glyphosphgate in the endosperm of unmodified wheat. The reduction in the level of the protein or gene expression or EPSPS protein affinity for glyphosate may occur at any stage in the development of the plant and grain, particularly in the meristem of the plant during vegetative growth and during the grain filling stage, or at all stages of the plant and grain development through to maturity.

As used herein, amino acid or nucleotide sequence "identity" and "similarity" are determined from an optimal global alignment between the two sequences being compared. An optimal global alignment is achieved using, for example, the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48:443-453). Sequences may also be aligned using algorithms known in the art including but not limited to CLUSTAL V algorithm or the Blastn or BLAST 2 sequence programs.

"Identity" means that an amino acid or nucleotide at a particular position in a first polypeptide or polynucleotide is identical to a corresponding amino acid or nucleotide in a second polypeptide or polynucleotide that is in an optimal global alignment with the first polypeptide or polynucleotide. In contrast to identity, "similarity" encompasses amino acids that are conservative substitutions. A "conservative" substitution is any substitution that has a positive score in the Blosum62 substitution matrix (Hentikoff and Hentikoff, 1992, Proc. Natl. Acad. Sci. USA 89: 10915-10919).

By the statement "sequence A is n % similar to sequence B," it is meant that n % of the positions of an optimal global alignment between sequences A and B consists of identical residues or nucleotides and conservative substitutions. By the statement "sequence A is n % identical to sequence B," it is meant that n % of the positions of an optimal global alignment between sequences A and B consists of identical residues or nucleotides.

As used herein, the term "gene" or "gene sequence" refers to the partial or complete coding sequence of a gene, its complement, and its 5' or 3' un-translated regions. A gene is also a functional unit of inheritance, and in physical terms is a particular segment or sequence of nucleotides along a molecule of DNA (or RNA, in the case of RNA viruses) involved in producing a polypeptide chain. The latter may be subjected to subsequent processing such as chemical modification or folding to obtain a functional protein or polypeptide. A gene may be isolated, partially isolated, or found within an organism's genome. By way of example, a transcription factor gene encodes a transcription factor polypeptide, which may be functional or require processing to function as an initiator of transcription.

Operationally, genes may be defined by the cis-trans test, a genetic test that determines whether two mutations occur in the same gene and that may be used to determine the

limits of the genetically active unit. A gene generally includes regions preceding (“leaders”; upstream) and following (“trailers”; downstream) the coding region. A gene may also include intervening, non-coding sequences, referred to as “introns”, located between individual coding segments, referred to as “exons.” Most genes have an associated promoter region, a regulatory sequence 5' of the transcription initiation codon (there are some genes that do not have an identifiable promoter). The function of a gene may also be regulated by enhancers, operators, and other regulatory elements.

As used herein, the term “modified plant” includes a plant that has a non-transgenic mutation, or a plant containing a transgene, or a plant that has undergone genomic editing or combinations thereof.

As used herein, the term “plant” includes reference to an immature or mature whole plant, including a plant from which seed or grain or anthers have been removed. A seed or embryo that will produce the plant is also considered to be the plant.

As used herein, the term “plant parts” includes plant protoplasts, plant cell tissue cultures from which wheat plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, ovules, pericarp, seed, flowers, florets, heads, spikes, leaves, roots, root tips, anthers, and the like.

As used herein, the term “polypeptide(s)” refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. “Polypeptide(s)” refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers, and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. “Polypeptide(s)” include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide.

As used herein, the term “polynucleotide(s)” generally refers to any polyribonucleotide or poly-deoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. This definition includes, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, cDNA, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. The term “polynucleotide(s)” also embraces short nucleotides or fragments, often referred to as “oligonucleotides,” that due to mutagenesis are not 100% identical but nevertheless code for the same amino acid sequence.

As used herein, the phrase “reduced affinity for glyphosate” refers to binding and/or interacting with glyphosate at a lower rate than normally measured. In one embodiment, a reduced affinity for glyphosate refers to a protein that shows no binding of glyphosate. In another embodiment, a reduced affinity for glyphosate refers to an EPSPS protein with one or more mutations that binds glyphosate at a slower rate than wild type EPSPS. In one embodiment, a reduced affinity for glyphosate refers to an EPSPS protein with one or more

mutations that binds glyphosate at a rate of about 10% or 20% or 30% or 40% or 50% or 60% or 70% or 80% or 85% or 90% or 95% of the wild type EPSPS binding rate for glyphosate. In one embodiment, a reduced affinity for glyphosate refers to an EPSPS protein with one or more mutations that binds glyphosate at a rate of less than 70%, or less than 60%, or less than 50%, or less than 40%, or less than 30%, or less than 20%, or less than 10%, or less than 5% of the wild type EPSPS binding rate for glyphosate.

As used herein, the term “resistance to glyphosate” is used interchangeably with the term “tolerance to glyphosate.” Both terms encompass a plant's ability to grow under an application rate of glyphosate that would normally cause harm and/or growth inhibition in a wild type plant. The term “resistance to glyphosate” encompasses a plant's ability to grow and/or produce at an application rate of glyphosate that would be detrimental or lethal to a wild type plant.

In one embodiment, resistance/tolerance to glyphosphate encompasses a plant's ability to grow, produce, or live in a higher percentage or amount of glyphosphate including but not limited to 1-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, 95-100% and greater than 100% as compared to a wild type plant.

In one embodiment, resistance/tolerance to glyphosphate encompasses a plant's ability to grow, produce, or live with an increased number of applications of glyphosphate as compared to a wild type plant including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 applications of glyphosphate.

A “reduced or non-functional fragment,” as is used herein, refers to a nucleic acid sequence that encodes a EPSPS protein that has reduced affinity for glyphosate as compared to the protein coding sequence of the whole nucleic acid sequence. In other words, it refers to a nucleic acid or fragment(s) thereof that substantially retains the capacity of encoding a EPSPS polypeptide, but the encoded EPSPS polypeptide has reduced affinity for glyphosate.

The term “fragment,” as used herein, refers to a polynucleotide sequence, (e.g. a PCR fragment) which is an isolated portion of the subject nucleic acid constructed artificially (e.g., by chemical synthesis) or by cleaving a natural product into multiple pieces, using restriction endonucleases or mechanical shearing, or a portion of a nucleic acid synthesized by PCR, DNA polymerase or any other polymerizing technique well known in the art, or expressed in a host cell by recombinant nucleic acid technology well known to one of skill in the art.

With reference to polynucleotides of the disclosure, the term “isolated polynucleotide” is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3'directions) in the naturally occurring genome of the organism from which it was derived. For example, the “isolated polynucleotide” may comprise a PCR fragment. In another embodiment, the “isolated polynucleotide” may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryote or eukaryote. An “isolated polynucleotide molecule” may also comprise a cDNA molecule.

As used herein, a single nucleotide polymorphism (SNP) is a single nucleotide base difference between two DNA according to nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G).

Single base variants are considered to be SNPs as are single base insertions and deletions (in/dels) in the genome.

As used herein, a “transgenic plant” refers to a plant that contains a gene construct (“transgene”) not found in a wild-type plant of the same species, variety or cultivar. A “transgene” as referred to herein has the normal meaning in the art of biotechnology and includes a genetic sequence that has been produced or altered by recombinant DNA or RNA technology and which has been introduced into the plant cell. The transgene may include genetic sequences derived from a plant cell. Typically, the transgene has been introduced into the plant by human manipulation such as, for example, by transformation but any method can be used as one of skill in the art recognizes.

As used herein, a “modified EPSPS gene” includes modification of the EPSPS gene through non-transgenic mutations or transgenes or genomic editing or combinations thereof.

As used herein, a “EPSPS derivative” refers to a EPSPS protein/peptide/polypeptide sequence that possesses reduced affinity for glyphosate as compared to the whole EPSPS protein/peptide/polypeptide sequence. The term “EPSPS derivative” encompasses the “fragments” or “chemical derivatives” of a modified EPSPS protein/peptide.

A wheat plant is defined herein as any plant of a species of the genus *Triticum*, which species is commercially cultivated, including, for example, *Triticum aestivum* L. ssp. *aestivum* (common or bread wheat), other subspecies of *Triticum aestivum*, *Triticum turgidum* L. ssp. *durum* (durum wheat, also known as macaroni or pasta wheat), *Triticum monococcum* L. ssp. *monococcum* (cultivated einkorn or small spelt), *Triticum timopheevi* ssp. *timopheevi*, *Triticum turgidum* L. ssp. *dicoccon* (cultivated emmer), and other subspecies of *Triticum turgidum* (Feldman). The wheat may be hexaploid wheat having an AABBDD type genome, or tetraploid wheat having an AABB type genome. Since genetic variation in wheat transferred to certain related species, including rye and barley by hybridization, the disclosure also includes the hybrid species thus formed, including triticale that is a hybrid between bread wheat and rye. In one embodiment, the wheat plant is of the species *Triticum aestivum*, and preferably of the subspecies *aestivum*. Alternatively, since mutations or transgenes can be readily transferred from *Triticum aestivum* to durum wheat, the wheat is preferably *Triticum turgidum* L. ssp. *durum*.

In another embodiment, the disclosure describes wheat plants exhibiting resistance to glyphosate as compared to wild type wheat plants without the inclusion of foreign nucleic acids in the wheat plant genome. In one embodiment, the disclosure relates to non-transgenic mutations in one or more EPSPS genes.

In still another embodiment, the disclosure relates to a series of independent human-induced non-transgenic mutations in one or more EPSPS genes; wheat plants having one or more of these mutations in at least one EPSPS gene thereof; and a method of creating and identifying similar and/or additional mutations in at least one EPSPS gene of wheat.

In yet another embodiment, the disclosure relates to a transgenic wheat plant with a transgene that reduces expression of the EPSPS gene and/or reduces affinity of the EPSPS protein for glyphosate as compared to a wild type plant.

In still another embodiment, the disclosure relates to wheat plant having a modified EPSPS gene, wherein the EPSPS gene is modified by genomic editing, and further

wherein said modification contributes to plants with resistance to glyphosate as compared to a wild type plant.

I. 5-enolpyruvylshikimate-3-phosphate Synthase (EPSPS)

In one embodiment, the disclosure relates to reducing expression of the EPSPS gene. In another embodiment, the disclosure relates to reducing affinity of the EPSPS protein for glyphosate. In one embodiment, the disclosure relates to wheat plants with an EPSPS protein with reduced affinity for glyphosate. In one embodiment, reducing expression of the EPSPS gene or reducing affinity of the EPSPS protein for glyphosate can be accomplished by non-transgenic mutations, transgenes, or genomic editing.

In one embodiment, the disclosure relates to modifying the EPSPS gene through non-transgenic mutations, or transgenes or genomic editing.

In one embodiment, the disclosure relates to modifying the EPSPS gene through non-transgenic mutations, or transgenes or genomic editing resulting in an altered EPSPS protein with reduced affinity for glyphosate but substantial affinity for plant substrates as compared to an unaltered or wild type EPSPS protein. In one embodiment, substantial affinity for plant substrates refers to an altered EPSPS protein that has at least 70%, or at least 75%, or at least 80% or at least 85% or at least 90% or at least 95% of the affinity of wild type EPSPS for plant substrates.

FIG. 1 provides an alignment of the protein sequence of rice EPSPS and the wheat EPSPS protein. As can be observed, substantial identity exists between the rice EPSPS and wheat EPSPS proteins. Minor differences can be found between the rice and wheat EPSPS protein in the N-terminal region.

The active region of the EPSPS protein is highly conserved between the rice and wheat EPSPS proteins. The active region is typically encompassed by the following amino acid sequence: FLGNAGTAMRPLTAAVVAAGGN (SEQ ID NO. 18).

II. Mutations of the EPSPS Gene

In one embodiment, wheat seeds can be mutagenized with ethyl methanesulfonate (EMS) and then grown into M1 plants. The M1 plants were then allowed to self-pollinate and seeds from the M1 plant were grown into M2 plants, which were then screened for mutations in their EPSPS loci. M1 plants can be screened for mutations but an advantage of screening the M2 plants is that all somatic mutations correspond to germline mutations. One of skill in the art would understand that a variety of wheat plant materials, including but not limited to, seeds, pollen, plant tissue or plant cells, may be mutagenized in order to create the EPSPS-mutated wheat plants of the disclosure. However, the type of plant material mutagenized may affect when the plant DNA is screened for mutations. For example, when pollen is subjected to mutagenesis prior to pollination of a non-mutagenized plant, the seeds resulting from that pollination are grown into M1 plants. Every cell of the M1 plants will contain mutations created in the pollen, thus these M1 plants may then be screened for EPSPS mutations instead of waiting until the M2 generation.

Mutagens that create primarily point mutations and short deletions, insertions, transversions, and or transitions (about 1 to about 5 nucleotides), such as chemical mutagens or radiation, may be used to create the mutations. Mutagens conforming with the method disclosed herein include, but

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are not limited to, ethyl methanesulfonate (EMS), methylmethane sulfonate (MMS), N-ethyl-N-nitrosourea (ENU), triethylmelamine (TEM), N-methyl-N-nitrosourea (MNU), procarbazine, chlorambucil, cyclophosphamide, diethyl sulfate, acrylamide monomer, melphalan, nitrogen mustard, vincristine, dimethylnitrosamine, N-methyl-N'-nitro-Nitrosoguanidine (MNNG), nitrosoguanidine, 2-aminopurine, 7, 12 dimethyl-benz(a)anthracene (DMBA), ethylene oxide, hexamethylphosphoramide, bisulfan, diepoxyalkanes (diepoxyoctane (DEO), diepoxybutane (BEB), and the like), 2-methoxy-6-chloro-9[3-(ethyl-2-chloro-ethyl)aminopropylamino] acridine dihydrochloride (ICR-170), and formaldehyde.

Any suitable method of plant DNA preparation now known or hereafter devised may be used to prepare the wheat plant DNA for EPSPS mutation screening. For example, see Chen & Ronald, *Plant Molecular Biology Reporter* 17:53-57, 1999; Stewart and Via, *Bio Techniques* 14:748-749, 1993. Additionally, several commercial kits are available, including kits from Qiagen (Valencia, Calif.) and Qbiogene (Carlsbad, Calif.).

In one embodiment, prepared DNA from individual wheat plants was then pooled in order to expedite screening for mutations in the EPSPS genes of the entire population of plants originating from the mutagenized plant tissue. The size of the pooled group may be dependent upon the sensitivity of the screening method used and the ploidy of the plants being screened. Preferably, groups of two or more individual wheat plants are pooled.

In another embodiment, after the DNA samples are pooled, the pools are subjected to EPSPS sequence-specific amplification techniques, such as Polymerase Chain Reaction (PCR). For a general overview of PCR, see *PCR Protocols: A Guide to Methods and Applications* (Innis, Gelfand, Sninsky, and White, eds.), Academic Press, San Diego, 1990. Any primer specific to an EPSPS locus or the sequences immediately adjacent to an EPSPS locus may be utilized to amplify the EPSPS sequences within the pooled DNA sample. Preferably, the primer is designed to amplify the regions of the EPSPS loci where useful mutations are most likely to arise. Most preferably, the primer is designed to amplify the conserved region of the EPSPS gene that codes for the enzyme's active domain. This region of the EPSPS enzyme is well known in the art (Padgett et al., *The Journal of Biological Chemistry* 266(33):22364-22369, 1991; Schonbrunn et al., *Proceedings of the National Academy of Sciences* 98(4):1376-1380, 2001; Funke et al., *The Journal of Biological Chemistry* 284(15):9854-9860, 2009). Additionally, it is preferable for the primer to avoid known polymorphic sites in order to ease screening for point mutations. In one embodiment, the primers are designed to amplify only one of the homoeologs so that only one homoeolog at a time is screened for mutations. The primers can be targeted to polymorphic sites between the homoeologs, so that they only amplify one of the homoeologs.

To facilitate detection of PCR products on a gel, the PCR primer may be labeled using any conventional or hereafter devised labeling method.

In one embodiment, *Oryza sativa* (rice) EPSPS cDNA (SEQ ID NO:1; NCBI Reference Sequence NM_001063247) and genomic DNA (SEQ ID NO: 2; NCBI Reference Sequence NC_008399) sequences were aligned against wheat expressed sequence tags (ESTs) in GenBank using NCBI's Basic Local Alignment Search Tool for nucleotides (BLASTN) and a Unigene cluster of ESTs (Ta. 12687; retired and replaced by Ta. 17912) was identified. The Unigene sequences were then aligned with the rice

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EPSPS cDNA and genomic DNA sequences and the PCR primers (SEQ ID NOs 4-5) shown in Table 1 were designed to amplify a region of the wheat EPSPS gene that coded for the active domain of the enzyme. These PCR primers were used to amplify EPSPS from wheat genomic DNAs that were prepared from the wild type tetraploid and hexaploid cultivars, Kronos (*Triticum turgidum*, *Durum*) and Express (*Triticum aestivum*, PVP #9000012), respectively.

TABLE 1

Exemplary Genomic Sequencing Primers			
SEQ ID	Primer Name	Primer ID	Sequence
4	TaEPS1CL	3155	ACAGTGAGGATGTCCACTACATGCTTGA
5	TaEPS1ER	3158	AAATAGCTCGCACTTGAGGCATCACCTT

PCR amplification products were cloned using a TOPO Ta Cloning® Kit (with pCR® 2.1-TOPO) (Invitrogen, Carlsbad, Calif. 92008) although any suitable cloning vector could be used. Multiple independent clones were sequenced. Two categories of sequences were identified in genomic DNA from Kronos. These two plus an additional sequence category were identified in Express genomic DNA. These were presumed to represent the three wheat EPSPS homoeologues. Based on an expected synteny with the rice genome, the wheat EPSPS A and D genome copies were expected to be localized on chromosomes 7A and 7D while the B genome copy was expected to be on chromosome 4A due to a known translocation of a portion of chromosome 7B (http://wheat.pw.usda.gov/NSF/project/mapping_data).

With this in mind, genomic DNAs were amplified from the Chinese Spring nullisomic/tetrasomic wheat lines containing altered chromosome complements of homoeologous groups 7 A, B, D and 4 A using the same PCR primers (SEQ ID NOs: 4-5) and the PCR amplification products were sequenced. The same three EPSPS sequence categories were identified in the nullisomic/tetrasomic lines as were identified in Express. The resulting sequences were determined to represent novel partial genomic sequences for the EPSPS homoeologous genes in wheat (SEQ ID NOs: 6-8).

In another embodiment, primers were then designed to amplify specifically each EPSPS homoeologue based upon nucleotide differences that were identified in the three partial genomic sequences. Exemplary PCR primers (SEQ ID NOs: 9-12) that have proven useful in identifying useful mutations within the EPSPS sequences are shown below in Table 2. The A homoeologue of EPSP was amplified using SEQ ID NO: 9 as the left primer in combination with SEQ ID NO: 10 as the right primer. The B homoeologue of EPSP was amplified using SEQ ID NO: 9 as the left primer in combination with SEQ ID NO: 11 as the right primer and this PCR product was further screened using the TaqMan protocol as described below. The D homoeologue of EPSP was amplified using SEQ ID NO: 9 as the left primer in combination with SEQ ID NO: 12 as the right primer.

TABLE 2

Exemplary Primers Useful for Detection of Preferred Mutations			
SEQ ID	Primer Name	Primer ID	Primer Sequence
10	TaEPS1CL	3155	ACAGTGAGGATGTCCACTACATGCTTGA
11	Ep486AR	3418	ACTTCTCTGACAGAGAACAGAAGTGTGCAC
12	Ep558BR	3419	TTGTGTAAGGTCGCATTGATCGTACTACCA
13	TaEpsJR	3223	GAAAACTAGAATCATGCTTTTGTACTCCACTATC

The PCR amplification products from the various primer combinations were sequenced to confirm that the primer pairs specifically amplified the desired homoeologues. The PCR products then were used to screen for EPSPS mutations in each homoeologue.

In another embodiment, the PCR amplification products may be screened for EPSPS mutations using any method that identifies nucleotide differences between wild type and mutant sequences. These may include, for example, without limitation, sequencing, denaturing high pressure liquid chromatography (dHPLC), constant denaturant capillary electrophoresis (CDCE), temperature gradient capillary electrophoresis (TGCE) (see Li et al., *Electrophoresis* 23(10):1499-1511, 2002), or by fragmentation using enzymatic cleavage, such as used in the high throughput method described by Colbert et al., *Plant Physiology* 126:480-484, 2001. Preferably the PCR amplification products are incubated with an endonuclease that preferentially cleaves mismatches in heteroduplexes between wild type and mutant sequences.

In another embodiment, cleavage products are electrophoresed using an automated sequencing gel apparatus, and gel images are analyzed with the aid of a standard commercial image-processing program. PCR amplification products may also be screened for single nucleotide polymorphisms using a TaqMan® SNP Genotyping Assay (Protocol Number 4332856C from Applied Biosystems, Foster City, Calif.).

TABLE 3

TaqMan Primers Useful for T173I Mutation Detection		
SEQ ID	Primer Name	Primer Sequence
14	HT_Tt◊I-Tt◊IF	ACGCCAAAGAGGAAGTAAAGCT
15	HT_Tt◊I-Tt◊IR	TCCAAACATACCCATGGATCTCATACT
16	HT_Tt◊I-Tt◊IV2 VIC	CGCATTGCAGTTCCA
17	HT_Tt◊I-Tt◊IM2 FAM	CATTGCAATTCCA

In one embodiment, mutations disclosed and contemplated herein include missense mutations in the active domains of the EPSPS enzymes that should decrease the binding of glyphosate with minimal alterations in the binding of the endogenous substrates, including phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid.

In one embodiment, mutations in wheat EPSPS gene include a mutation that results in a change from threonine to isoleucine at amino acid position 173 (referred to herein as

the T173I mutation), a mutation that results in a change from proline to serine at amino acid position 177 (referred to herein as the P177S mutation), and a mutation that results in a change from proline to leucine at amino acid position 177 (referred to herein as the P177L mutation) For clarity, the mutated amino acids in the wheat EPSPS protein are numbered according to the published rice EPSPS protein sequence (SEQ ID NO: 3). Preferred mutations also include other mutations in the EPSPS enzymes' active domain that alter enzyme activity as described above. Each novel mutation is evaluated in order to predict its impact on protein function (i.e., completely tolerated to loss-of-function) using bioinformatics tools such as SIFT (Sorting Intolerant from Tolerant; Ng and Henikoff, *Nucleic Acids Research* 31:3812-3814, 2003), PSSM (Position-Specific Scoring Matrix; Henikoff and Henikoff, *Computer Applications in the Biosciences* 12:135-143, 1996) and PARSESNP (Taylor and Greene, *Nucleic Acids Research* 31:3808-3811, 2003). For example, a SIFT score that is less than 0.05 and a large change in PSSM score (e.g., roughly 10 or above) indicate a mutation that is likely to have a deleterious effect on protein function.

A. EPSPS Gene

In one embodiment, the disclosure relates to one or more non-transgenic mutations in the EPSPS gene. In another embodiment, the disclosure relates to one or more mutations in the EPSPS gene. In one embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations.

In another embodiment, the EPSPS gene may contain one or more non-transgenic mutations that results in a polypeptide having one or more mutations recited in Table 4 and corresponding mutations in homoeologues and combinations thereof.

In another embodiment, the disclosure relates to corresponding mutations to the one or more non-transgenic mutations disclosed herein in the EPSPS gene in a corresponding homoeologue. By way of example, an identified mutation in the EPSPS gene of the A genome may be a beneficial mutation in the EPSPS gene of the B and/or D genome. One of ordinary skill in the art will understand that the mutation in the homoeologue may not be in the exact same location.

One of ordinary skill in the art understands that there may be natural variation in the genetic sequences of the EPSPS genes in different wheat varieties.

The inventors have determined that to achieve plants with resistance to glyphosate, mutations that alter EPSPS gene function are desirable. Preferred mutations include missense and nonsense changes, including mutations that prematurely truncate the translation of one or more EPSPS proteins from messenger RNA, such as those mutations that create a stop

codon within the coding region of an EPSPS messenger RNA. Such mutations include insertions, repeat sequences, splice junction mutations, modified open reading frames (ORFs) and point mutations.

In still another embodiment, one or more mutations are in the EPSPS gene of the A genome. In another embodiment, one or more mutations are in the EPSPS gene of the B genome. In still another embodiment, one or more mutations are in the EPSPS gene of the D genome. In yet another embodiment, one or more mutations are in the EPSPS genes of the A and B genomes. In still another embodiment, one or more mutations are in the EPSPS genes of the A and D genomes. In another embodiment, one or more mutations are in the EPSPS genes of the B and D genomes. In yet another embodiment, one or more mutations are in the EPSPS genes of the A, B, and D genomes.

1. A Genome

In one embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the A

genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations. In one embodiment, one or more non-transgenic mutations are in both alleles of the EPSPS gene in the A genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the A genome. In one embodiment, the mutations are homozygous.

The following mutations identified in Table 4 are exemplary of the mutations created and identified according to various embodiments disclosed herein. They are offered by way of illustration, not limitation. It is to be understood that the mutations below are merely exemplary and that similar mutations are also contemplated.

Table 4 provides a list of representative mutations in the EPSPS gene in the A, B, and D genomes. Amino acid changes are identified according to both the published rice protein sequence, SEQ ID NO: 3, as well as the wheat protein sequence, SEQ ID NO: 9.

TABLE 4

Representative mutations in the Wheat EPSPS gene in the A, B, and D genomes.						
Cultivar	Genome	Amino Acid Change (SEQ ID NO: 3)	Amino Acid Change (SEQ ID NO: 9)	PSSM Score	SIFT Score	DNA Mutation Score
Express	A	M175I	M170I	7.6	0.44	Missense
Express	A	P177S	P172S	10.3	0.02	Severe Missense
Express	A	A181T	A176T	6.0	0.06	Missense
Express	A	A171V	A166V	5.5	0.20	Missense
Express	A	R176W	R171W	28.0	0.00	Severe Missense
Express	A	G169S	G164S	15	0	Severe Missense
Express	A	A185T	A180T		0.38	Missense
Express	B	A174V	A169V	5.5	0.20	Missense
Express	B	T179M	T174M	18.3	0.01	Severe Missense
Express	B	V182I	V177I	14.2	0.02	Severe Missense
Express	B	A181V	A176V	8.4	0.1	Missense
Express	B	A184T	A179T	16.3	0.04	Severe Missense
Express	B	G172R	G167R	17	0	Severe Missense
Express	B	T173I	T168I	13.1	0.05	Severe Missense
Express	B	G169S	G164S	15	0	Severe Missense
Express	B	A171T	A166T	13	0	Severe Missense
Express	B	M175I	M171I	10.6	0.08	Missense
Express	B	A180T	A175T	21.7	0	Severe Missense
Express	B	V196L	V191L	18.1	0.04	Severe Missense
Express	B	R198K	R193K	20	0.01	Severe Missense
Express	B	M199I	M194I	24	0	Severe Missense
Express	B	V209I	V204I	8.4	0	Severe Missense
Express	B or D	M175I	M171I	11.0	0.09	Missense
Express	D	T173I	T168I	13.9	0.00	Severe Missense
Express	B	P177L	P172L			
Kronos	A	M175I	M171I	7.6	0.44	Missense
Kronos	A	G195D	G190D	26.9	0	Severe Missense
Kronos	B	A184T	A179T	2.7	0.35	Missense
Express	A	P177S-A	P172S-A			
		T173I-A	T168I-A			
Express	D	P177S-D	P172S-D			
		T173I-D	T168I-D			
Express	A and D	P177S-A	P172S-A			
		T173I-A	T168I-A			
		P177S-D	P172S-D			
		T173I-D	T168I-D			
Express	A and D	P177S-A	P172S-A			
		T173I-A	T168I-A			
		T173I-D	T168I-D			
Express	A and D	P177S-A	P172S-A			
		P177S-D	P172S-D			
		T173I-D	T168I-D			
Express	A, B, and D	P177S-A	P172S-A			
		T173I-A	T168I-A			
		T173I-B	T168I-B			
		P177S-D	P172S-D			
		T173I-D	T168I-D			
Express	A, B, and D	P177S-A	P172S-A			
		T173I-A	T168I-A			
		T173I-B	T168I-B			
		T173I-D	T168I-D			

TABLE 4-continued

Representative mutations in the Wheat EPSPS gene in the A, B, and D genomes.						
Cultivar	Genome	Amino Acid Change (SEQ ID NO: 3)	Amino Acid Change (SEQ ID NO: 9)	PSSM Score	SIFT Score	DNA Mutation Score
Express	A, B, and D	T173I-A	T168I-A			
		T173I-B	T168I-B			
		P177S-D	P172S-D			
		T173I-D	T168I-D			
Express	A, B, and D	T173I-A	T168I-A			
		P177S-A	P172S-A			
		P177L-B	P172L-B			
		P177S-D	P172S-D			
		T173I-D	T168I-D			

In one embodiment, two or more mutations identified in the A genome and recited in Table 4 can be combined so that the A genome contains two or more mutations.

In another embodiment, two or more mutations identified in the B genome and recited in Table 4 can be combined so that the B genome contains two or more mutations.

In yet another embodiment, two or more mutations identified in the D genome and recited in Table 4 can be combined so that the D genome contains two or more mutations.

TABLE 5

Representative combination mutations in Wheat EPSPS gene of the A, B, and D genomes.				
Cultivar	Genome	Mutation (location on SEQ ID NO: 3)	Mutation (location on SEQ ID NO: 9)	
Express	A	P177S -A	P172S -A	
		T173I -A	T168I -A	
Express	D	P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		T173I -D	T168I -D	
Express	A and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A, B, and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		T173I -B	T168I -B	
		P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A, B, and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		T173I -B	T168I -B	
		T173I -D	T168I -D	
Express	A, B, and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		T173I -B	T168I -B	
		P177S -D	P172S -D	
Express	A, B, and D	P177S -A	P172S -A	
		T173I -A	T168I -A	
		T173I -B	T168I -B	
		P177S -D	P172S -D	
		T173I -D	T168I -D	

TABLE 5-continued

Representative combination mutations in Wheat EPSPS gene of the A, B, and D genomes.				
Cultivar	Genome	Mutation (location on SEQ ID NO: 3)	Mutation (location on SEQ ID NO: 9)	
Express	A, B, and D	T173I -A	T168I -A	
		T173I -B	T168I -B	
		P177S -D	P172S -D	
		T173I -D	T168I -D	
Express	A, B, and D	P177S -A	P172S -A	
		P177L -B	P172L -B	
		T173I -D	T168I -D	

In one embodiment, any mutations identified in the A genome in Table 4 can be combined with any mutation in the B genome recited in Table 4.

TABLE 6

Representative combinations of wheat plants with mutations in the EPSPS gene in both the A and B genomes. Amino acid designation corresponds to the amino acid position in SEQ ID NO: 9.			
Cultivar	A Genome	B genome	
Express/Kronos	P172S	T174M	
Express/Kronos	P172S	V177I	
Express/Kronos	P172S	A176V	
Express/Kronos	P172S	A179T	
Express/Kronos	P172S	G167R	
Express/Kronos	P172S	T168I	
Express/Kronos	P172S	G164S	
Express/Kronos	P172S	A166T	
Express/Kronos	P172S	M171I	
Express/Kronos	P172S	A175T	
Express/Kronos	P172S	V191L	
Express/Kronos	P172S	R193K	
Express/Kronos	P172S	M194I	
Express/Kronos	P172S	V205I	
Express/Kronos	P172S	M170I	
Express/Kronos	M170I	T168I	
Express/Kronos	P172S	T168I	
Express/Kronos	A176T	T168I	
Express/Kronos	A166V	T168I	
Express/Kronos	R171W	T168I	
Express/Kronos	G164S	T168I	
Express/Kronos	A181T	T168I	

In another embodiment, any mutations identified in the A genome in Table 4 can be combined with any mutation in the D genome recited in Table 4.

TABLE 7

Representative combinations of wheat plants with mutations in the EPSPS gene of the A and D genomes. Amino acid designation corresponds to the amino acid position in SEQ ID NO: 9.		
Cultivar	A Genome	D genome
Express/Kronos	P172S	T168I
Express/Kronos	M170I	T168I
Express/Kronos	P172S	T168I
Express/Kronos	A176T	T168I
Express/Kronos	A166V	T168I
Express/Kronos	R171W	T168I
Express/Kronos	G164S	T168I
Express/Kronos	A180T	T168I

In yet another embodiment, any mutations identified in the A genome in Table 4 can be combined with any mutation in the B genome in Table 4 and any mutation in the D genome recited in Table 4.

TABLE 8

Representative combinations of wheat plants with mutations in the EPSPS gene in the A, B, and D genomes. Amino acid designation corresponds to the amino acid position in SEQ ID NO: 9.			
Cultivar	A Genome	B genome	D genome
Express/Kronos	P172S	T174M	T168I or M170I
Express/Kronos	P172S	V177I	T168I or M170I
Express/Kronos	P172S	A176V	T168I or M170I
Express/Kronos	P172S	A179T	T168I or M170I
Express/Kronos	P172S	G167R	T168I or M170I
Express/Kronos	P172S	T168I	T168I or M170I
Express/Kronos	P172S	G164S	T168I or M170I
Express/Kronos	P172S	A166T	T168I or M170I
Express/Kronos	P172S	M170I	T168I or M170I
Express/Kronos	P172S	A175T	T168I or M170I
Express/Kronos	P172S	V191L	T168I or M170I
Express/Kronos	P172S	R193K	T168I or M170I
Express/Kronos	P172S	M194I	T168I or M170I
Express/Kronos	P172S	V204I	T168I or M170I
Express/Kronos	P172S	M170I	T168I or M170I
Express/Kronos	M170I	T168I	T168I or M170I
Express/Kronos	P172S	T168I	T168I or M170I
Express/Kronos	A166T	T168I	T168I or M170I
Express/Kronos	A166V	T168I	T168I or M170I
Express/Kronos	R171W	T168I	T168I or M170I
Express/Kronos	G164S	T168I	T168I or M170I
Express/Kronos	A180T	T168I	T168I or M170I

In another embodiment, any mutations identified in the B genome in Table 4 can be combined with any mutation in the D genome recited in Table 4.

In one embodiment, the disclosure relates to a polynucleotide of the EPSPS gene in the A genome corresponding to SEQ ID NO: 6 with one or more non-transgenic mutations that produces a polypeptide with one or more mutations recited in Table 4. In another embodiment, the polynucleotide that codes for a polypeptide with one or more non-transgenic mutations listed in Table 4 has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to SEQ ID NO: 6.

In still another embodiment, the polynucleotide codes for an EPSPS protein with one or more non-transgenic mutation listed in Table 4, wherein the EPSPS protein comprises one or more non-transgenic mutations and has 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to the EPSPS-A protein.

2. B Genome

In one embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the B genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations. In one embodiment, one or more non-transgenic mutations are in both alleles of the EPSPS gene in the B genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the B genome. In still another embodiment, the mutations are homozygous.

In one embodiment, the disclosure relates to a polynucleotide of the EPSPS gene in the B genome corresponding to SEQ ID NO: 7 with one or more non-transgenic mutations that codes for a polypeptide with one or more mutations listed in Table 4. In another embodiment, the polynucleotide with one or more non-transgenic mutations codes for a polypeptide having one or more mutations recited in Table 4, and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to SEQ ID NO: 7.

In still another embodiment, the disclosure relates to a polynucleotide of the EPSPS gene in the B genome with one or more non-transgenic mutation that codes for a polypeptide with one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to the EPSPS-B protein.

3. D Genome

In one embodiment, the disclosure relates to multiple non-transgenic mutations in the EPSPS gene of the D genome including but not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and greater than 10 mutations. In one embodiment, one or more non-transgenic mutations are in both alleles of the EPSPS gene in the D genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the D genome. In still another embodiment, the mutations are homozygous.

In one embodiment, the disclosure relates to a polynucleotide of the EPSPS gene in the D genome corresponding to SEQ ID NO: 8 with one or more non-transgenic mutations that codes for a polypeptide with one or more mutations listed in Table 4. In another embodiment, the polynucleotide with one or more non-transgenic mutations codes for a polypeptide having one or more mutations recited in Table 4, and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to SEQ ID NO: 8.

In still another embodiment, the disclosure relates to a polynucleotide of the EPSPS gene in the D genome with one or more non-transgenic mutation that codes for a polypeptide with one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity to the EPSPS-D protein.

B. EPSPS Proteins

In yet another embodiment, the disclosure relates to one or more non-transgenic mutations in the EPSPS gene (as discussed above in the section entitled EPSPS Mutations) that result in a EPSPS protein with one or more mutations as compared to wild type EPSPS protein. In one embodiment, the non-transgenic mutations include but are not limited to the mutations recited in Table 4, corresponding mutations in homoeologues, and combinations thereof.

In another embodiment, the disclosure relates to one or more non-transgenic mutations in the EPSPS gene that results in an EPSPS protein with reduced affinity for glyphosate. In some embodiments, a mutation in the EPSPS gene reduces expression of the EPSPS protein. In other

1. Expression Level of EPSPS Protein

In another embodiment, the expression level of EPSPS protein with one or more mutations disclosed herein is reduced by 0-2%, 2-5%, 5-7%, 7-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, and 95-99% of the expression level of the wild type EPSPS protein.

In still another embodiment, the expression level of EPSPS protein with one or more mutations disclosed herein is reduced from 10-20%, or from 20-30%, or from 30-40%, or from 40-50%, or from 50-60%, or from 60-70%, or from 70-80%, or from 80-90%, or from 90-99% as compared to the expression level of the wild type EPSPS protein.

2. Activity of EPSPS Protein

In yet another embodiment, the EPSPS protein with one or more mutations disclosed herein has reduced affinity for glyphosate, wherein the reduced affinity is 0-1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% and by more than 99% of the affinity of the wild type EPSPS protein for glyphosate. In another embodiment, the EPSPS protein with one or more mutations disclosed herein has no affinity or zero affinity for glyphosate as compared to wild type EPSPS protein.

In yet another embodiment, the EPSPS protein with one or more mutations disclosed herein has reduced affinity for glyphosate but wild type affinity for plant or endogenous substrates. In one embodiment, the EPSPS protein with one or more mutations disclosed herein has substantial affinity for plant or endogenous substrates, wherein the affinity for plant substrates is about 100%, or 99%, or 98%, or 97%, or 96%, or 95%, or 94%, or 93%, or 92%, or 91%, or 90% as compared to wild type EPSPS protein.

In yet another embodiment, the activity of the EPSPS protein with one or more mutations disclosed herein is from 1-10% or from 10-30% or from 30-50% or from 50-70% or from 70-80% or from 80-90% or from 90-95% of the activity level of the wild type EPSPS protein.

III. Genomic Editing

In one embodiment, the disclosure relates to a plant with reduced expression of the EPSPS gene and/or reduced activity of the EPSPS protein, wherein reduced expression of the EPSPS gene and/or reduced activity of the EPSPS protein is achieved by genomic editing.

In one embodiment, the disclosure relates to a wheat plant with a genomically edited EPSPS gene, wherein the wheat plant has an altered EPSPS protein compared to a wild type plant.

Genome editing, or genome editing with engineered nucleases (GEEN), is a type of genetic engineering in which DNA is inserted, replaced, or removed from a genome using artificially engineered nucleases, or "molecular scissors." The nucleases create specific double-stranded breaks (DSBs) at desired locations in the genome, and harness the

cell's endogenous mechanisms to repair the induced break by natural processes of homologous recombination (HR) and nonhomologous end-joining (NHEJ). There are currently four main families of engineered nucleases being used: Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, and engineered meganuclease with a re-engineered homing endonucleases.

A. Zinc Finger Nucleases (ZFNs)

Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes. By taking advantage of endogenous DNA repair machinery, these reagents can be used to precisely alter the genomes of higher organisms.

ZFNs consist of an engineered zinc finger DNA-binding domain fused to the cleavage domain of the FokI restriction endonuclease. ZFNs can be used to induce double-stranded breaks (DSBs) in specific DNA sequences and thereby promote site-specific homologous recombination with an exogenous template. The exogenous template contains the sequence that is to be introduced into the genome.

Publicly available methods for engineering zinc finger domains include: (1) Context-dependent Assembly (CoDA), (2) Oligomerized Pool Engineering (OPEN), and (3) Modular Assembly.

In one embodiment, the disclosure relates to increasing the glyphosate tolerance of the EPSPS gene and its encoded enzyme using ZFNs.

B. Transcription Activator-Like Effector Nucleases (TALENs)

TALEN is a sequence-specific endonuclease that consists of a transcription activator-like effector (TALE) and a FokI endonuclease. TALE is a DNA-binding protein that has a highly conserved central region with tandem repeat units of 34 amino acids. The base preference for each repeat unit is determined by two amino acid residues called the repeat-variable di-residue (RVD), which recognizes one specific nucleotide in the target DNA. Arrays of DNA-binding repeat units can be customized for targeting specific DNA sequences. As with ZFNs, dimerization of two TALENs on targeted specific sequences in a genome results in FokI-dependent introduction of DSBs, stimulating homology directed repair (HDR) and Non-homologous end joining (NHEJ) repair mechanisms.

In one embodiment, the disclosure relates to increasing the glyphosate tolerance of the EPSPS gene and its encoded enzyme using TALENs.

C. CRISPR/Cas System

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system is an RNA-Guided Endonuclease technology for genome engineering. There are two distinct components to this system: (1) a guide RNA and (2) an endonuclease, in this case the CRISPR associated (Cas) nuclease, Cas9.

The guide RNA is a combination of the endogenous bacterial crRNA and tracrRNA into a single chimeric guide RNA (gRNA) transcript. The gRNA combines the targeting specificity of the crRNA with the scaffolding properties of the tracrRNA into a single transcript. When the gRNA and the Cas9 are expressed in the cell, the genomic target sequence can be modified or permanently disrupted.

The gRNA/Cas9 complex is recruited to the target sequence by the base-pairing between the gRNA sequence and the complementarity to the target sequence in the

genomic DNA. For successful binding of Cas9, the genomic target sequence must also contain the correct Protospacer Adjacent Motif (PAM) sequence immediately following the target sequence. The binding of the gRNA/Cas9 complex localizes the Cas9 to the genomic target sequence so that the wild-type Cas9 can cut both strands of DNA causing a Double Strand Break (DSB). Cas9 will cut 3-4 nucleotides upstream of the PAM sequence. A DSB can be repaired through one of two general repair pathways: (1) NHEJ DNA repair pathway or (2) the HDR pathway. The NHEJ repair pathway often results in insertions/deletions (InDels) at the DSB site that can lead to frameshifts and/or premature stop codons, effectively disrupting the open reading frame (ORE) of the targeted gene.

The HDR pathway requires the presence of a repair template, which is used to fix the DSB. HDR faithfully copies the sequence of the repair template to the cut target sequence. Specific nucleotide changes can be introduced into a targeted gene by the use of HDR with a repair template.

In one embodiment, the disclosure relates to increasing the glyphosate tolerance of the EPSPS gene and its encoded enzyme using the CRISPR/cas9 system.

D. Meganuclease with Re-Engineered Homing Nuclease

Meganucleases are endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs); as a result this site generally occurs only once in any given genome. For example, the 18-base pair sequence recognized by the I-SceI meganuclease would on average require a genome twenty times the size of the human genome to be found once by chance (although sequences with a single mismatch occur about three times per human-sized genome). Meganucleases are therefore considered to be the most specific naturally occurring restriction enzymes.

Among meganucleases, the LAGLIDADG family of homing endonucleases has become a valuable tool for the study of genomes and genome engineering over the past fifteen years. By modifying their recognition sequence through protein engineering, the targeted sequence can be changed.

In one embodiment, the disclosure relates to increasing the glyphosate tolerance of the EPSPS gene and its encoded enzyme using a meganuclease with a re-engineered homing nuclease.

IV. Wheat Cultivars

In one embodiment, a wheat cultivar having at least one EPSPS gene that is diploid, polyploid, tetraploid, and hexaploid may be used.

In another embodiment, the wheat is *Triticum aestivum*.

In one embodiment, any cultivar of wheat can be used to create mutations in an EPSPS gene. In one embodiment, any cultivar of wheat can be used to create mutations in the EPSPS gene of the A genome. In another embodiment, any cultivar of wheat can be used to create mutations in the EPSPS gene of the B genome. In another embodiment, any cultivar of wheat can be used to create mutations in the EPSPS gene of the D genome.

In one embodiment, any cultivar of wheat can be used as lines to cross EPSPS mutations into different cultivars. In another embodiment, any cultivar of wheat having at least one EPSPS gene may be used including but not limited to hard red spring wheat, hard white wheat, durum wheat, soft white spring wheat, soft white winter wheat, hard red winter

wheat, common wheat, club wheat, spelt wheat, emmer wheat, pasta wheat and *turgidum* wheat.

In one embodiment, hard red spring wheat includes but is not limited to Bullseye, Cabernet, Cal Rojo, Hank, Joaquin, Kelse, Lariat, Lassik, Malbec, Mika, PR 1404, Redwing, Summit 515, SY 314, Triple IV, Ultra, WB-Patron, WB-Rockland, Yecora Rojo, Accord, Aim, Anza, Baker, Beth Hashita, Bonus, Borah, Brim, Brooks, Buck Pronto, Butte 86, Cavalier, Challenger, Chief, Ciano T79, Colusa, Companion, Copper, Cuyama, Dash 12, Eldon, Enano, Express, Expresso, Jefferson, Genero F81, Grandin, Helena 554, Hollis, Imuris T79, Inia 66R, Jerome, Kern, Len, Marshall, McKay, Nomad, Northwest 10, Oslo, Pavon F76, Pegasus, Pitic 62, Poco Red, Powell, Probrand 711, Probrand 751, Probrand 771, Probrand 775, Probred, Printa Queguay, Printa Quintal, Rich, RSI 5, Sagittario, Scarlet, Serra, Shasta, Solano, Spillman, Sprite, Stander, Stellar, Stoa, Success, Summit, Sunstar 2, Sunstar King, Tadinia, Tammy, Tanori 71, Tara 2000, Tempo, Tesia T79, Topic, UI Winchester, Vance, Vandal, W444, Wampum, Wared, WB-Fuzion, Westbred 906R, Westbred 911, Westbred 926, Westbred 936, Westbred Discovery, Westbred Rambo, Yolo, and Zeke.

In another embodiment, hard white wheat includes but is not limited to Blanca Fuerte, Blanca Grande 515, Blanca Royale, Clear White, Patwin, Patwin 515, WB-Cristallo, WB-Paloma, WB-Perla, Alta Blanca, Blanca Grande, Delano, Golden Spike, ID377S, Klasic, Lochsa, Lolo, Macon, Otis, Phoenix, Pima 77, Plata, Pristine, Ramona 50, Siete Cerros 66, Vaiiolet, and Winsome.

In yet another embodiment, durum wheat includes but is not limited to Crown, Desert King, Desert King HP, Duraking, Fortissimo, Havasu, Kronos, Maestrale, Normanno, Orita, Platinum, Q-Max, RSI 59, Saragolla, Tango, Tipai, Topper, Utopia, Volante, WB-Mead, Westmore, Aldente, Aldura, Altar 84, Aruba, Bittern, Bravadur, Candura, Cortez, Deluxe, Desert Titan, Durex, Durfort, Eddie, Germaines 5003D, Imperial, Kofa, Levante, Matt, Mead, Mexicali 75, Minos, Modoc, Mohawk, Nudura, Ocotillo, Produra, Reva, Ria, Septre, Sky, Tacna, Titan, Trump, Ward, Westbred 803, Westbred 881, Westbred 883, Westbred 1000D, Westbred Laker, Westbred Turbo, and Yavaros 79.

In another embodiment, soft white spring wheat includes but is not limited to Alpowa, Alturas, Babe, Diva, JD, New Dirkwin, Nick, Twin, Whit, Blanca, Bliss, Calorwa, Centennial, Challis, Dirkwin, Eden, Edwall, Fielder, Fieldwin, Jubilee, Louise, Owens, Penawawa, Pomerelle, Sterling, Sunstar Promise, Super Dirkwin, Treasure, UI Cataldo, UI Pettit, Urquie, Vanna, Waduel, Waduel 94, Wakanz, Walladay, Wawawai, Whitebird, and Zak.

In still another embodiment, soft white winter wheat includes but is not limited to AP Badger, AP Legacy, Brundage 96, Bruneau, Cara, Goetze, Legion, Mary, Skiles, Stephens, SY Ovation, Tubbs, WB-Junction, WB-528, Xerpha, Yamhill, Barbee, Basin, Bitterroot, Bruehl, Castan, Chukar, Coda, Daws, Edwin, Eltan, Faro, Finch, Foote, Gene, Hill 81, Hiller, Hubbard, Hyak, Hyslop, Idaho 587, Kmor, Lambert, Lewjain, MacVicar, Madsen, Malcolm, Masami, McDermid, Moro, Nugaines, ORCF-101, ORCF-102, ORCF-103, Rod, Rohde, Rulo, Simon, Salute, Temple, Tres, Tubbs 06, UICF-Brundage, WB-523, and Weatherford.

In another embodiment, hard red winter wheat includes but is not limited to Andrews, Archer, Batum, Blizzard, Bonneville, Boundary, Declo, Deloris, Finley, Garland, Hatton, Hoff, Longhorn, Manning, Meridian, Promontory, Vona, Wanser, Winridge.

In another embodiment, common wheat (hexaploid, free threshing), *Triticum aestivum* ssp *aestivum* includes but is not limited to Sonora, Wit Wolkoring, Chiddam Blanc De Mars, India-Jammu, Foisy.

In still another embodiment, spelt wheat (hexaploid, not free threshing), *Triticum aestivum* ssp *spelta* includes but is not limited to Spanish Spelt, Swiss Spelt.

In yet another embodiment, Emmer Wheat (tetraploid), *Triticum turgidum* ssp. *dicoccum* includes but is not limited to Ethiopian Blue Tinge.

In another embodiment, pasta wheat (tetraploid, free threshing), *Triticum turgidum* ssp *durum* includes but is not limited to Blue Beard, Durum-Iraq.

In yet another embodiment, *Turgidum* Wheat (tetraploid, free threshing), *Triticum turgidum* ssp *turgidum* includes but is not limited to Akmolinka, Maparcha.

In one embodiment, a cultivar of wheat having at least one EPSPS gene with substantial percent identity to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO. 8 may be used with the methods and compositions disclosed herein.

As used herein with regard to the wheat cultivars, "substantial percent identity" means that the DNA sequence of the gene is sufficiently similar to SEQ ID NO: 6, 7, and 8 at the nucleotide level to code for a substantially similar protein, allowing for allelic differences (or alternate mRNA splicing) between cultivars. In one embodiment, "substantial percent identity" may be present when the percent identity in the coding region between the EPSPS gene and SEQ ID NO: 6, 7, and 8 is as low as about 85%, provided that the percent identity in the conserved regions of the gene is higher (e.g., at least about 90%). Preferably the percent identity in the coding region is 85-90%, more preferably 90-95%, and optimally, it is above 95%. Thus, one of skill in the art may prefer to utilize a wheat cultivar having commercial popularity or one having specific desired characteristics in which to create the EPSPS-mutated wheat plants, without deviating from the scope and intent of the disclosure. Alternatively, one of skill in the art may prefer to utilize a wheat cultivar having few polymorphisms, such as an in-bred cultivar, in order to facilitate screening for mutations within one or more EPSPS genes in accordance with the disclosure

V. Representative Methodology for Identification of EPSPS Mutations

One of ordinary skill in the art will appreciate that numerous techniques and methods are available for generating mutations and/or non-transgenic mutations. One representative methodology is described below.

In order to create and identify the EPSPS mutations and wheat plants disclosed herein, a method known as TILLING was utilized. See McCallum et al., *Nature Biotechnology* 18:455-457, 2000; McCallum et al., *Plant Physiology*, 123: 439-442, 2000; U.S. Publication No. 20040053236; and U.S. Pat. No. 5,994,075, all of which are incorporated herein by reference. In the basic TILLING methodology, plant materials, such as seeds, are subjected to chemical mutagenesis, which creates a series of mutations within the genomes of the seeds' cells. The mutagenized seeds are grown into adult M1 plants and self-pollinated. DNA samples from the resulting M2 plants are pooled and are then screened for mutations in a gene of interest. Once a mutation is identified in a gene of interest, the seeds of the M2 plant carrying that mutation are grown into adult M3 plants and screened for the phenotypic characteristics associated with that mutation in the gene of interest.

In one embodiment, the tetraploid cultivar Kronos was used. In other embodiments, the hexaploid cultivar Express was used.

In one embodiment, seeds from wheat are mutagenized and then grown into M1 plants. The M1 plants are then allowed to self-pollinate and seeds from the M1 plant are grown into M2 plants, which are then screened for mutations in their EPSPS loci. While M1 plants can be screened for mutations in accordance with alternative embodiments disclosed herein, one advantage of screening the M2 plants is that all somatic mutations correspond to germline mutations.

One of skill in the art will understand that a variety of wheat plant materials, including, but not limited to, seeds, pollen, plant tissue or plant cells, may be mutagenized in order to create the EPSPS-mutated wheat plants disclosed herein. However, the type of plant material mutagenized may affect when the plant DNA is screened for mutations. For example, when pollen is subjected to mutagenesis prior to pollination of a non-mutagenized plant, the seeds resulting from that pollination are grown into M1 plants. Every cell of the M1 plants will contain mutations created in the pollen, thus these M1 plants may then be screened for EPSPS mutations instead of waiting until the M2 generation.

Mutagens that create primarily point mutations and short deletions (about 1 to about 30 nucleotides), insertions, transversions, and or transitions, such as chemical mutagens or radiation, such as x-rays and fast neutrons, may be used to create the mutations. Mutagens conforming with the methods disclosed herein include, but are not limited to, ethyl methanesulfonate (EMS), methylmethane sulfonate (MMS), N-ethyl-N-nitrosourea (ENU), triethylmelamine (TEM), N-methyl-N-nitrosourea (MNU), procarbazine, chlorambucil, cyclophosphamide, diethyl sulfate, acrylamide monomer, melphalan, nitrogen mustard, vincristine, dimethylnitrosamine, N-methyl-N'-nitro-Nitrosoguanidine (MNNG), nitrosoguanidine, 2-aminopurine, 7, 12 dimethylbenz(a)anthracene (DMBA), ethylene oxide, hexamethylphosphoramide, bisulfan, diepoxyalkanes (diepoxyoctane (DEO), diepoxybutane (DEB), and the like), 2-methoxy-6-chloro-9[3-(ethyl-2-chloro-ethyl)aminopropylamino] acridine dihydrochloride (ICR-170), sodium azide, and formaldehyde. Spontaneous mutations in a EPSPS gene that may not have been directly caused by the mutagen can also be identified.

Any suitable method of plant DNA preparation now known or hereafter devised may be used to prepare the wheat plant DNA for EPSPS mutation screening. For example, see Chen & Ronald, *Plant Molecular Biology Reporter* 17:53-57, 1999; Stewart and Via, *Bio Techniques* 14:748-749, 1993. Additionally, several commercial kits designed for this purpose are available, including kits from Qiagen (Valencia, Calif.) and Qbiogene (Carlsbad, Calif.).

In one embodiment, prepared DNA from individual wheat plants are pooled in order to expedite screening for mutations in one or more EPSPS genes of the entire population of plants originating from the mutagenized plant tissue. The size of the pooled group may be dependent upon the sensitivity of the screening method used. Preferably, groups of two or more individual wheat plants are pooled.

In another embodiment, after the DNA samples are pooled, the pools are subjected to EPSPS sequence-specific amplification techniques, such as Polymerase Chain Reaction (PCR). For a general overview of PCR, see *PCR Protocols: A Guide to Methods and Applications* (Innis, Gelfand, Sninsky, and White, eds.), Academic Press, San Diego, 1990.

Any primer specific to a EPSPS locus or the sequences immediately adjacent to one of these loci may be utilized to amplify the EPSPS sequences within the pooled DNA sample. Preferably, the primer is designed to amplify the regions of the EPSPS locus where useful mutations are most likely to arise. Most preferably, the primer is designed to detect exonic regions of one or more EPSPS genes. Additionally, it is preferable for the primer to target known polymorphic sites to design genome specific primers in order to ease screening for point mutations in a particular genome. To facilitate detection of PCR products on a gel, the PCR primer may be labeled using any conventional or hereafter devised labeling method.

In one embodiment, primers are designed based upon the EPSPS genes (SEQ ID NOs: 6, 7, and 8). In another embodiment, primers can be designed that are 5' or 3' to the EPSPS genes.

In another embodiment, the PCR amplification products may be screened for EPSPS mutations using any method that identifies nucleotide differences between wild type and mutant sequences. These may include, for example, without limitation, sequencing, denaturing high pressure liquid chromatography (dHPLC), constant denaturant capillary electrophoresis (CDCE), temperature gradient capillary electrophoresis (TGCE) (see Li et al., *Electrophoresis* 23(10):1499-1511, 2002), or by fragmentation using enzymatic cleavage, such as used in the high throughput method described by Colbert et al., *Plant Physiology* 126:480-484, 2001. Preferably, the PCR amplification products are incubated with an endonuclease that preferentially cleaves mismatches in heteroduplexes between wild type and mutant sequences.

In another embodiment, cleavage products are electrophoresed using an automated sequencing gel apparatus, and gel images are analyzed with the aid of a standard commercial image-processing program.

In yet another embodiment, once an M2 plant having a mutated EPSPS gene sequence is identified, the mutations are analyzed to determine their effect on the expression, translation, and/or activity of a EPSPS enzyme. In one embodiment, the PCR fragment containing the mutation is sequenced, using standard sequencing techniques, in order to determine the exact location of the mutation in relation to the overall EPSPS sequence. Each mutation is evaluated in order to predict its impact on protein function (i.e., from completely tolerated to causing loss-of-function) using bioinformatics tools such as SIFT (Sorting Intolerant from Tolerant; Ng and Henikoff, *Nucleic Acids Research* 31:3812-3814, 2003), PSSM (Position-Specific Scoring Matrix; Henikoff and Henikoff, *Computer Applications in the Biosciences* 12:135-143, 1996) and PARSESNP (Taylor and Greene, *Nucleic Acids Research* 31:3808-3811, 2003). For example, a SIFT score that is less than 0.05 and a large change in PSSM score (e.g., roughly 10 or above) indicate a mutation that is likely to have a deleterious effect on protein function. These programs are known to be predictive, and it is understood by those skilled in the art that the predicted outcomes are not always accurate.

In another embodiment, if the initial assessment of a mutation in the M2 plant indicates it to be of a useful nature and in a useful position within a EPSPS gene, then further phenotypic analysis of the wheat plant containing that mutation may be pursued. In hexaploid wheat, mutations in each of the A, B and D genomes usually must be combined before a phenotype can be detected. In tetraploid wheat, A and B genome mutations are combined. In addition, the mutation containing plant can be backcrossed or outcrossed two times or more in order to eliminate background muta-

tions at any generation. Then, the backcrossed or outcrossed plant can be self-pollinated or crossed in order to create plants that are homozygous for the EPSPS mutations.

Several physical characteristics of these homozygous EPSPS mutant plants are assessed to determine if the mutation results in a useful phenotypic change in the wheat plant without resulting in undesirable negative effects, such as significantly reduced seed yields.

In one embodiment, seeds of the plant containing multiple preferred single mutations in EPSPS can be re-mutagenized and then the progeny can be screened to find the preferred second-site mutation that increases the glyphosate tolerance above the tolerance of the single-site mutations.

VI. Methods of Producing a Wheat Plant

In another embodiment, the disclosure relates to a method for producing a wheat plant with resistance to glyphosate. In another embodiment, the disclosure relates to a method for producing a wheat plant with resistance to glyphosate.

In another embodiment, the disclosure relates to a method of out-crossing EPSPS gene mutations to wild type plants.

In still another embodiment, the disclosure relates to a method for producing a plant having one or more EPSPS proteins with reduced affinity for glyphosate as compared to the wild type EPSPS protein.

In one embodiment, the method comprises inducing at least one non-transgenic mutation in at least one copy of a EPSPS gene in plant material or plant parts from a parent plant; growing or using the mutagenized plant material to produce progeny plants; analyzing mutagenized plant material and/or progeny plants to detect at least one mutation in at least one copy of a EPSPS gene; and selecting progeny plants that have at least one mutation in at least one copy of a EPSPS gene.

In another embodiment, the method further comprises crossing progeny plants that have at least one mutation in at least one copy of a EPSPS gene with other progeny plants that have at least one mutation in a different copy of a EPSPS gene. The process of identifying progeny plants with mutations and crossing said progeny plants with other progeny plants, which have mutations, can be repeated to produce progeny wheat plants with reduced EPSPS activity.

In another embodiment, the affinity of the altered EPSPS protein in the wheat plant for glyphosate is reduced to 0-2%, 2-5%, 5-7%, 7-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, or 95-99% of the affinity of EPSPS protein in the wild type plant.

A. Methods of Producing a Plant with One or More Mutations in the EPSPS Gene in More than One Genome

In still another embodiment, the disclosure relates to a method for producing a plant comprising the following: inducing at least one non-transgenic mutation in at least one copy of a EPSPS gene in plant material from a parent plant that comprises a mutation in a EPSPS gene; growing or using the mutagenized plant material to produce progeny plants; and selecting progeny wheat plants that have at least one mutation in at least two copies of a EPSPS gene. For example, the parent plant may have a mutation in a EPSPS gene of the A genome. The selected progeny plants may have a mutation in a EPSPS gene of the A genome and one or more mutations in the EPSPS gene of the B genome. This example is provided merely for clarification and should not limit the methods disclosed herein.

In yet another embodiment, the disclosure relates to a method for producing a plant comprising inducing at least

one non-transgenic mutation in at least one copy of a EPSPS gene in plant material from a parent plant that comprises at least one mutation in two EPSPS genes; growing or using the mutagenized plant material to produce progeny plants; and selecting progeny plants that have at least one mutation in three copies of a EPSPS gene. In this embodiment, there would be at least one mutation in the EPSPS gene of the A, B and D genomes.

In another embodiment, the disclosure relates to a method for producing a wheat plant comprising crossing a first plant that has at least one non-transgenic mutation in a first EPSPS gene with a second plant that has at least one non-transgenic mutation in a second EPSPS gene; and selecting progeny plants that have at least one mutation in at least two copies of a EPSPS gene.

In another embodiment, the disclosure relates to a method for producing a plant comprising crossing a first plant that has at least one non-transgenic mutation in a first and second EPSPS gene with a second plant that has at least one non-transgenic mutation in a third EPSPS gene; and selecting progeny plants that have at least one mutation in all three copies of a EPSPS gene. In this embodiment, there would be at least one mutation in the EPSPS gene of the A, B, and D genomes.

VII. Wheat Plant, Wheat Seed and Parts of Wheat Plant

In one embodiment, a wheat plant with resistance to glyphosate is produced according to the methods disclosed herein. In yet another embodiment, a wheat plant with resistance to glyphosate and unaltered growth characteristics as compared to a wild type wheat plant is produced according to the methods disclosed herein. In yet another embodiment, a wheat plant with resistance to glyphosate and wild type germination rates is disclosed herein.

In yet another embodiment, a wheat plant with resistance to glyphosate that produces seed that germinates is disclosed herein. In yet another embodiment, a wheat plant with resistance to glyphosate and wild type fertility is disclosed herein.

In yet another embodiment, a wheat plant with resistance to glyphosate and normal yields is disclosed herein.

In one embodiment, wheat plants or parts thereof are tolerant to an application rate of 34.4 g or more, or 68.8 g or more, of the isopropylamine salt of glyphosate per hectare in the field.

In another embodiment, the wheat plant, wheat seed or parts of a wheat plant have one or more mutations in a EPSPS gene or a modified EPSPS gene. In another embodiment, the wheat plant, wheat seed or parts of a wheat plant have one or more mutations in EPSPS genes.

In another embodiment, the disclosure relates to a wheat plant, wheat seed or parts of a wheat plant comprising one or more non-transgenic mutations in the EPSPS gene. In another embodiment, the disclosure relates to a wheat plant, wheat seed or parts of a wheat plant comprising at least one non-transgenic mutation in the EPSPS gene in each of two genomes. In still another embodiment, the disclosure relates to a wheat plant, wheat seed or parts of a wheat plant comprising at least one non-transgenic mutation in the EPSPS gene in each of three genomes.

In one embodiment, the wheat plant, wheat seed or parts of a wheat plant comprises one or more non-transgenic mutations in both alleles of the EPSPS gene in the A

genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the A genome.

In one embodiment, the wheat plant, wheat seed or parts of a wheat plant comprises one or more non-transgenic mutations in both alleles of the EPSPS gene in the B genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the B genome.

In one embodiment, the wheat plant, wheat seed or parts of a wheat plant comprises one or more non-transgenic mutations in both alleles of the EPSPS gene in the D genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the D genome.

In another embodiment, the wheat plant, wheat seed or parts of the wheat plant comprise a polynucleotide that codes for a polypeptide with one or more non-transgenic mutations listed in Table 4, wherein the polynucleotide has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to SEQ ID NO: 6.

In still another embodiment, the wheat plant, wheat seed or parts of a wheat plant comprise a polynucleotide that codes for a polypeptide with one or more non-transgenic mutations listed in Table 4, wherein the polypeptide comprises one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to the EPSPS-A protein.

In another embodiment, the wheat plant, wheat seed or parts of the wheat plant comprise a polynucleotide having one or more mutations that codes for a polypeptide with one or more non-transgenic mutations listed in Table 4, wherein the polynucleotide has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to SEQ ID NO: 7.

In still another embodiment, the wheat plant, wheat seed or parts of a wheat plant comprise a polynucleotide with one or more non-transgenic mutations that codes for a EPSPS protein, wherein the EPSPS protein comprises one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to the EPSPS-B protein.

In another embodiment, the wheat plant, wheat seed or parts of the wheat plant comprise a polynucleotide having one or more mutations that codes for a polypeptide with one or more non-transgenic mutations listed in Table 4, wherein the polynucleotide has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to SEQ ID NO: 8.

In still another embodiment, the wheat plant, wheat seed or parts of a wheat plant comprise a polynucleotide with one or more non-transgenic mutations that codes for a EPSPS protein, wherein the EPSPS protein comprises one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to the EPSPS-D protein.

In another embodiment, the wheat plant, wheat seed or parts of a wheat plant has one or more mutations in the EPSPS gene that codes for a polypeptide having one or more mutations enumerated in Table 4 and corresponding mutations in the homoeologues. A wheat plant, wheat seed or parts of a wheat plant can be generated having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

23, 24, 25 or greater than 25 of the mutations disclosed herein including but not limited to the mutations disclosed in Table 4, as well as mutations in corresponding homoeologues.

VIII. Grain, Flour and Starch

In another embodiment, the disclosure relates to a wheat grain, flour or starch comprising one or more non-transgenic mutations in the EPSPS gene or a modified EPSPS gene. In another embodiment, the disclosure relates to wheat grain comprising an embryo, wherein the embryo comprises one or more non-transgenic mutations in a EPSPS gene or a modified EPSPS gene.

In another embodiment, the wheat grain, flour or starch comprises one or more non-transgenic mutations in the EPSPS genes including but not limited to the mutations recited in Table 4 and the corresponding mutations in homologues, and homoeologues.

In still another embodiment, the disclosure relates to a wheat grain or flour comprising at least one non-transgenic mutation in the EPSPS gene in one, two, or three genomes.

In still another embodiment, the disclosure relates to a wheat grain, flour, or starch comprising one or more non-transgenic mutations in the EPSPS gene of the A genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the A genome.

In one embodiment, the wheat grain, flour, or starch comprises one or more non-transgenic mutations in both alleles of the EPSPS gene in the B genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the B genome.

In one embodiment, the wheat grain, flour or starch comprises one or more non-transgenic mutations in both alleles of the EPSPS gene in the D genome. In another embodiment, the non-transgenic mutations are identical in both alleles of the EPSPS gene of the D genome.

In one embodiment, the disclosure relates to wheat grain, wheat flour or starch comprising a polynucleotide of the EPSPS gene in the A genome corresponding to SEQ ID NO: 6 with one or more non-transgenic mutations that codes for a polypeptide having one or more mutations listed in Table 4. In another embodiment, the wheat grain or wheat flour comprise a polynucleotide with one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to SEQ ID NO: 6.

In still another embodiment, wheat grain, wheat flour or starch comprise a polynucleotide with one or more non-transgenic mutations that codes for a EPSPS protein having one or more mutations recited in Table 4, wherein the EPSPS protein has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to the EPSPS-A protein.

In one embodiment, the disclosure relates to wheat grain, wheat flour or starch comprising a polynucleotide of the EPSPS gene in the B genome corresponding to SEQ ID NO: 7 with one or more mutations that codes for a polypeptide having one or more mutations recited in Table 4. In another embodiment, the wheat grain or wheat flour comprise a polynucleotide with one or more non-transgenic mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to SEQ ID NO: 7

In still another embodiment, wheat grain, wheat flour or starch comprise a polynucleotide with one or more non-transgenic mutations that codes for a EPSPS protein having

one or more mutations recited in Table 4, wherein the EPSPS protein has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to EPSPS-B protein.

5 In one embodiment, the disclosure relates to wheat grain, wheat flour or starch comprising a polynucleotide of the EPSPS gene in the D genome corresponding to SEQ ID NO: 8 with one or more mutations that codes for a polypeptide having one or more mutations listed in Table 4. In another
10 embodiment, the wheat grain or wheat flour comprise a polynucleotide with one or more mutations and has 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or
15 similarity to SEQ ID NO: 8.

In still another embodiment, wheat grain, wheat flour or starch comprise a polynucleotide with one or more mutations that codes for a EPSPS protein having one or more mutations recited in Table 4, wherein the EPSPS protein has
20 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% identity or similarity to EPSPS-D protein.

In still another embodiment, the disclosure relates to wheat grain or flour comprising an endosperm and a reduced gene expression level, activity or expression level and activity of the EPSPS gene as compared to wild type wheat grain or flour.

IX. Food Products

In one embodiment, the disclosure is directed to a flour or other product produced from the grain or flour discussed above. In another embodiment, the flour, the coarse fraction or purified starch may be a component of a food product.

35 The food product includes but is not limited to a bagel, a biscuit, a bread, a bun, a croissant, a dumpling, an English muffin, a muffin, a pita bread, a quickbread, a flat bread, a sourdough bread, a refrigerated/frozen dough product, dough, baked beans, a burrito, chili, a taco, a tamale, a
40 tortilla, a pot pie, a ready to eat cereal, a ready to eat meal, stuffing, a microwaveable meal, a brownie, a cake, a cheese-cake, a coffee cake, a cookie, a dessert, a pastry, a sweet roll, a candy bar, a pie crust, pie filling, baby food, a baking mix, a batter, a breading, a gravy mix, a meat extender, a meat
45 substitute, a seasoning mix, a soup mix, a gravy, a roux, a salad dressing, a soup, sour cream, a noodle, a pasta, ramen noodles, chow mein noodles, lo mein noodles, an ice cream inclusion, an ice cream bar, an ice cream cone, an ice cream
50 sandwich, a cracker, a crouton, a doughnut, an egg roll, an extruded snack, a fruit and grain bar, a microwaveable snack product, a nutritional bar, a pancake, a par-baked bakery product, a pretzel, a pudding, a granola-based product, a snack chip, a snack food, a snack mix, a waffle, a pizza crust,
55 animal food or pet food.

In one embodiment, the flour is a whole grain flour (ex.—an ultrafine-milled whole grain flour, such as an ultrafine-milled whole grain wheat flour). In one embodiment, the whole grain flour includes a refined flour constituent (ex.—refined wheat flour or refined flour) and a coarse fraction (ex.—an ultrafine-milled coarse fraction). Refined wheat flour may be flour which is prepared, for example, by grinding and bolting (sifting) cleaned wheat. The Food and Drug Administration (FDA) requires flour to meet certain
60 particle size standards in order to be included in the category of refined wheat flour. The particle size of refined wheat flour is described as flour in which not less than 98% passes

through a cloth having openings not larger than those of woven wire cloth designated "212 micrometers (U.S. Wire 70)."

In another embodiment, the coarse fraction includes at least one of: bran and germ. For instance, the germ is an embryonic plant found within the wheat kernel. The germ includes lipids, fiber, vitamins, protein, minerals and phytonutrients, such as flavonoids. The bran may include several cell layers and has a significant amount of lipids, fiber, vitamins, protein, minerals and phytonutrients, such as flavonoids.

For example, the coarse fraction or whole grain flour or refined flour of the disclosure may be used in various amounts to replace refined or whole grain flour in baked goods, snack products, and food products. The whole grain flour (i.e.—ultrafine-milled whole grain flour) may also be marketed directly to consumers for use in their homemade baked products. In an exemplary embodiment, a granulation profile of the whole grain flour is such that 98% of particles by weight of the whole grain flour are less than 212 micrometers.

In another embodiment, the whole grain flour or coarse fraction or refined flour may be a component of a nutritional supplement. The nutritional supplement may be a product that is added to the diet containing one or more ingredients, typically including: vitamins, minerals, herbs, amino acids, enzymes, antioxidants, herbs, spices, probiotics, extracts, prebiotics and fiber.

In a further embodiment, the nutritional supplement may include any known nutritional ingredients that will aid in the overall health of an individual, examples include but are not limited to vitamins, minerals, other fiber components, fatty acids, antioxidants, amino acids, peptides, proteins, lutein, ribose, omega-3 fatty acids, and/or other nutritional ingredients. Because of the high nutritional content of the endosperm, there may be many uses that confer numerous benefits to an individual, including, delivery of fiber and other essential nutrients, increased digestive function and health, weight management, blood sugar management, heart health, diabetes risk reduction, potential arthritis risk reduction, and overall health and wellness for an individual.

In still another embodiment, the whole grain flour or coarse fraction or refined flour may be a component of a dietary supplement. The Code of Federal Regulations defines a dietary supplement as a product that is intended to supplement the diet and contains one or more dietary ingredients including: vitamins, minerals, herbs, botanicals, amino acids, and other substances or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled on the front panel as being a dietary supplement.

In yet another embodiment, the whole grain flour or coarse fraction or refined flour may be a fiber supplement or a component thereof. The fiber supplement may be delivered in, but is not limited to the following forms: instant beverage mixes, ready-to-drink beverages, nutritional bars, wafers, cookies, crackers, gel shots, capsules, chews, chewable tablets, and pills. One embodiment delivers the fiber supplement in the form of a flavored shake or malt type beverage.

In another embodiment, the whole grain flour or coarse fraction or refined flour may be included as a component of a digestive supplement. The whole grain flour or coarse fraction or refined flour may be a component of a digestive supplement alone or in combination with one or more prebiotic compounds and/or probiotic organisms. Prebiotic compounds are non-digestible food ingredients that may beneficially affect the host by selectively stimulating the

growth and/or the activity of a limited number of microorganisms in the colon. Examples of prebiotic compounds within the scope of the disclosure, may include, but are not limited to: oligosaccharides and inulins.

Probiotics are microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Probiotic organisms include, but are not limited to: *Lactobacillus*, *Bifidobacteria*, *Escherichia*, *Clostridium*, *Lactococcus*, *Streptococcus*, *Enterococcus*, and *Saccharomyces*.

In yet another embodiment, the whole grain flour or coarse fraction or refined flour may be included as a component of a functional food. The Institute of Food Technologists defines functional foods as, foods and food components that provide a health benefit beyond basic nutrition. This includes conventional foods, fortified, enriched, or enhanced foods, and dietary supplements. The whole grain flour and coarse fraction or refined flour include numerous vitamins and minerals, have high oxygen radical absorption capacities, and are high in fiber, making them ideally suited for use in/as a functional food.

In another embodiment, the whole grain flour or coarse fraction or refined flour may be used in medical foods. Medical food is defined as a food that is formulated to be consumed or administered entirely under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. The nutrient contents and antioxidant capacities of the whole grain flour and coarse fraction or refined flour make them ideal for use in medical foods.

In yet another embodiment, the whole grain flour or coarse fraction or refined flour may also be used in pharmaceuticals. The whole grain flour and coarse fraction or refined flour are high in fiber and have a very fine granulation making them suitable for use as a carrier in pharmaceuticals.

In still another embodiment, delivery of the whole grain flour or coarse fraction or refined flour as a nutritional supplement, dietary supplement or digestive supplement is contemplated via delivery mechanisms where the whole grain flour or coarse fraction is the single ingredient or one of many nutritional ingredients. Examples of delivery mechanisms include but are not limited to: instant beverage mixes, ready-to-drink beverages, nutritional bars, wafers, cookies, crackers, gel shots, capsules, and chews.

In yet another embodiment, a milling process may be used to make a multi-wheat flour, or a multi-grain coarse fraction. In one embodiment, bran and germ from one type of wheat may be ground and blended with ground endosperm or whole grain wheat flour of another type of wheat. Alternatively bran and germ of one type of grain may be ground and blended with ground endosperm or whole grain flour of another type of grain.

In still another embodiment, bran and germ from a first type of wheat or grain may be blended with bran and germ from a second type of wheat or grain to produce a multi-grain coarse fraction. It is contemplated that the disclosure encompasses mixing any combination of one or more of bran, germ, endosperm, and whole grain flour of one or more grains. This multi-grain, multi-wheat approach may be used to make custom flour and capitalize on the qualities and nutritional contents of multiple types of grains or wheats to make one flour.

The whole grain flour disclosed herein may be produced via a variety of milling processes. One exemplary process involves grinding grain in a single stream without separating

endosperm, bran, and germ of the grain into separate streams. Clean and tempered grain is conveyed to a first passage grinder, such as a hammermill, roller mill, pin mill, impact mill, disc mill, air attrition mill, gap mill, or the like.

After grinding, the grain is discharged and conveyed to a sifter. Any sifter known in the art for sifting a ground particle may be used. Material passing through the screen of the sifter is the whole grain flour of the disclosure and requires no further processing. Material that remains on the screen is referred to as a second fraction. The second fraction requires additional particle reduction. Thus, this second fraction may be conveyed to a second passage grinder.

After grinding, the second fraction may be conveyed to a second sifter. Material passing through the screen of the second sifter is the whole grain flour. The material that remains on the screen is referred to as the fourth fraction and requires further processing to reduce the particle size. The fourth fraction on the screen of the second sifter is conveyed back into either the first passage grinder or the second passage grinder for further processing via a feedback loop.

It is contemplated that the whole grain flour, coarse fraction, purified starch and/or grain products of the disclosure may be produced by a number of milling processes known in the art.

X. Plant Breeding

In another embodiment, the disclosure is directed to methods for plant breeding using wheat plants and plant parts with one or more non-transgenic mutations in the EPSPS gene.

One such embodiment is the method of crossing a wheat variety with one or more non-transgenic mutations in the EPSPS gene with another variety of wheat to form a first generation population of F1 plants. The population of first generation F1 plants produced by this method is also an embodiment of the disclosure. This first generation population of F1 plants will comprise an essentially complete set of the alleles of a wheat variety with one or more non-transgenic mutations in the EPSPS gene. One of ordinary skill in the art can utilize either breeder books or molecular methods to identify a particular F1 plant produced using a wheat variety with one or more non-transgenic mutations in the EPSPS gene, and any such individual plant is also encompassed by this disclosure. These embodiments also cover use of transgenic or backcross conversions of wheat varieties with one or more mutations in the EPSPS gene to produce first generation F1 plants.

In another embodiment, the disclosure relates to a method of developing a progeny wheat plant. A method of developing a progeny wheat plant comprises crossing a wheat variety with one or more non-transgenic mutations in the EPSPS gene with a second wheat plant and performing a breeding method. A specific method for producing a line derived from a wheat variety with one or more non-transgenic mutations in the EPSPS gene is as follows.

One of ordinary skill in the art would cross a wheat variety with one or more non-transgenic mutations in the EPSPS gene with another variety of wheat, such as an elite variety. The F1 seed derived from this cross would be grown to form a homogeneous population. The F1 seed would contain one set of the alleles from a wheat variety with one or more non-transgenic mutations in the EPSPS gene and one set of the alleles from the other wheat variety.

The F1 genome would be made-up of 50% of a wheat variety with one or more non-transgenic mutations in the EPSPS gene and 50% of the other elite variety. The F1 seed

would be grown to form F2 seed. The F1 seed could be allowed to self, or bred with another wheat cultivar.

On average the F2 seed would have derived 50% of its alleles from a wheat variety with one or more non-transgenic mutations in the EPSPS gene and 50% from the other wheat variety, but various individual plants from the population would have a much greater percentage of their alleles derived from a wheat variety with one or more non-transgenic mutations in the EPSPS gene (Wang J. and R. Bernardo, 2000, *Crop Sci.* 40:659-665 and Bernardo, R. and A. L. Kahler, 2001, *Theor. Appl. Genet.* 102:986-992).

The F2 seed would be grown and selection of plants would be made based on visual observation and/or measurement of traits and/or marker assisted selection. The wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived progeny that exhibit gene-derived traits would be selected and each plant would be harvested separately. This F3 seed from each plant would be grown in individual rows and allowed to self. Then selected rows or plants from the rows would be harvested and threshed individually. The selections would again be based on visual observation and/or measurements for desirable traits of the plants, such as one or more of the desirable wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived traits.

The process of growing and selection would be repeated any number of times until a homozygous wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived wheat plant is obtained. The homozygous wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived wheat plant would contain desirable traits derived from the wheat variety with one or more non-transgenic mutations in the EPSPS gene, some of which may not have been expressed by the other original wheat variety to which the wheat variety with one or more non-transgenic mutations in the EPSPS gene was crossed and some of which may have been expressed by both wheat varieties but now would be at a level equal to or greater than the level expressed in the wheat variety with one or more non-transgenic mutations in the EPSPS gene.

The breeding process, of crossing, selfing, and selection may be repeated to produce another population of wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived wheat plants with, on average, 25% of their genes derived from wheat variety with one or more non-transgenic mutations in the EPSPS gene, but various individual plants from the population would have a much greater percentage of their alleles derived from the wheat variety with one or more non-transgenic mutations in the EPSPS gene. Another embodiment of the disclosure is a homozygous wheat variety with one or more non-transgenic mutations in the EPSPS gene-derived wheat plant that has been crossed with another wheat plant with one or more non-transgenic mutations in the EPSPS gene-derived traits.

A. Mutations as Markers

Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a plant, a tissue, a cell, a nucleus, a chromosome or a gene. Genetic markers used in genetics and plant breeding can be classified into two categories: classical markers and DNA markers. Classical markers include morphological markers, cytological markers and biochemical markers. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting—nuclear acid hybridization,

PCR—polymerase chain reaction, and DNA sequencing), such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), etc.

SNPs provide the ultimate/simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and thus they can provide the maximum number of markers. SNPs occur very commonly in animals and plants. Typically, SNP frequencies are in a range of one SNP every 100-300 base pairs in plants. SNPs may be present within coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes at different frequencies in different chromosome regions.

SNPs are co-dominant markers, often linked to genes and present in the simplest/ultimate form for polymorphism, and thus they have become very attractive and potential genetic markers in genetic study and breeding. Moreover, SNPs can be very easily automated and quickly detected, with a high efficiency for detection of polymorphisms.

In one embodiment, the disclosure relates to mutations in the EPSPS gene, which are single nucleotide polymorphisms, that can be used as markers in plant breeding. The mutations in the EPSPS gene are causative and their segregation can be followed using, for example, KASP probes.

In another embodiment, mutations identified in Section II of this disclosure can be used as markers in plant breeding. In yet another embodiment, one or more mutations in Tables 1-3 can be used as markers in plant breeding.

In one embodiment, the mutations can be followed using techniques including but not limited to SNP-Restriction Fragment Length Polymorphism (RFLP); CAPS; Axiom SNP Arrays; iSelect Array; TaqMan Probes, and KASP Probes. In another embodiment, Next Generation Sequencing techniques can be used including but not limited to 454 Life Sciences (Roche Applied Science, Indianapolis, Ind.); HiSeq (Illumina, San Diego, Calif.); SOLiD and Ion Torrent (Life Technologies Corporation, Carlsbad, Calif.).

PCR-based KASP™ genotyping assay is a homogeneous, fluorescence (FRET) based assay that enables accurate bi-allelic discrimination of known SNPs and InDels. A key feature of PCR-based KASP technology is the use of a universal FRET cassette reporter system that eliminates the need for costly dual-labelled probes. The allele-specific forward primers each have a proprietary tail sequence that corresponds with one of two FRET cassettes: one label with FAM dye and the other with HEX dye. Bi-allelic discrimination is achieved through the competitive binding of the two allele-specific forward primers.

The plants, compositions and methods are further described by the following non-limiting paragraphs:

1. A wheat plant comprising a mutation in a EPSPS gene in at least one of the A, B, or D genomes, wherein the mutation contributes to a wheat plant having resistance to glyphosate as compared to a wild type plant.

2. A wheat plant comprising a mutation in an EPSPS gene in at least one of the A, B, or D genomes, wherein the gene mutation produces an EPSPS protein with a mutation in the active region, and further wherein the mutation contributes to wheat plant having resistance to glyphosate as compared to a wild type plant.

3. A wheat plant comprising a mutation in a EPSPS gene in at least one of the A, B, or D genomes, wherein the gene mutation produces an EPSPS protein with reduced affinity for glyphosate and similar affinity as wild type for plant

substrates, and further wherein the mutation contributes to a wheat plant having resistance to glyphosate as compared to a wild type plant

4. The wheat plant of any of paragraphs 1-3, wherein the mutation in the EPSPS gene is in the B and D genomes.

5. The wheat plant of any of paragraphs 1-3, wherein the mutation in the EPSPS gene is in the A and B genomes.

6. The wheat plant of any of paragraphs 1-3, wherein the mutation in the EPSPS gene is in the A and D genomes.

7. The wheat plant of any of paragraphs 1-3, wherein the mutation in the EPSPS gene is in the A, B, and D genomes.

8. The wheat plant of any of the preceding paragraphs, wherein the mutation results in an EPSPS protein with reduced affinity for glyphosate.

9. The wheat plant of any of the preceding paragraphs, wherein the mutation results in an EPSPS protein with similar affinity as wild type for plant substrates.

10. The wheat plant of any of the preceding paragraphs, wherein the mutation results in an EPSPS protein with reduced affinity for glyphosate and similar affinity as wild type for plant substrates.

11. The wheat plant of any of the preceding paragraphs, wherein the mutation results in an EPSPS protein with affinity for glyphosate selected from the group consisting of: about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80, or 90% of the affinity of wild type EPSPS for glyphosate.

12. The wheat plant of any of the preceding paragraphs, wherein said wheat plant is homozygous for the mutation.

13. The wheat plant of any of the preceding paragraphs, which is *Triticum aestivum* ssp. *aestivum*.

14. The wheat plant of any of the preceding paragraphs, which is *Triticum turgidum* subsp. *Durum*.

15. The wheat plant of any of the preceding paragraphs, wherein the mutation is recited in Tables 1-3.

16. Wheat grain from the wheat plant of any of the preceding paragraphs.

17. Flour comprising the wheat grain of any of the preceding paragraphs.

18. A food product comprising a component of the wheat plant of any of the preceding paragraphs.

19. A wheat seed, plant part or progeny thereof from the wheat plant of any of the preceding paragraphs.

20. A transgenic wheat plant comprising a transgene that reduces expression of a EPSPS gene and/or reduces affinity of an EPSPS protein for glyphosate, wherein the reduced expression and/or reduced affinity for glyphosate contributes to a wheat plant having resistance to glyphosate.

21. The wheat plant of paragraph 20, wherein the transgene results an EPSPS protein with similar affinity as wild type for plant substrates.

22. The wheat plant of paragraph 20, wherein the transgene results in an EPSPS protein with a mutation in the active region of the protein.

23. The wheat plant of paragraph 20, wherein the transgene results in an EPSPS protein with reduced affinity for glyphosate and similar affinity as wild type EPSPS for plant substrates.

24. The wheat plant of paragraph 20, wherein the transgene results in an EPSPS protein with affinity for glyphosate selected from the group consisting of: about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80, or 90% of the affinity of wild type EPSPS for glyphosate.

25. The wheat plant of paragraph 20, which is *Triticum aestivum* ssp. *aestivum*.

26. The wheat plant of paragraph 20, which is *Triticum turgidum* subsp. *Durum*.

27. Wheat grain from the wheat plant of any of paragraphs 20-26.

28. Flour comprising the wheat grain of paragraph 27.

29. A food product comprising a component of the wheat plant of paragraphs 20-28.

30. A wheat seed, plant part or progeny thereof from the wheat plant of paragraphs 20-26.

31. A wheat plant comprising a modified EPSPS gene, wherein the EPSPS gene was modified by genomic editing, and said modification contributes to said wheat plant having resistance to glyphosate.

32. The wheat plant of paragraph 31, wherein the modified EPSPS gene results an EPSPS protein with wild type affinity for plant substrates.

33. The wheat plant of paragraph 31, wherein the modified EPSPS gene results in an EPSPS protein with a mutation in the active region of the protein.

34. The wheat plant of paragraph 31, wherein the modified EPSPS gene results in an EPSPS protein with reduced affinity for glyphosate and similar affinity as wild type for plant substrates.

35. The wheat plant of paragraph 31, wherein the modified EPSPS gene results in an EPSPS protein with affinity for glyphosate selected from the group consisting of: about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80, or 90% of the affinity of wild type EPSPS for glyphosate.

36. The wheat plant of paragraph 31, which is *Triticum aestivum* ssp. *aestivum*.

37. The wheat plant of paragraph 31, which is *Triticum turgidum* subsp. *Durum*.

38. Wheat grain from the wheat plant of any of paragraphs 31-37.

39. Flour comprising the wheat grain of paragraph 38.

40. A food product comprising a component of the wheat plant of paragraphs 31-39.

41. A wheat seed, plant part or progeny thereof from the wheat plant of paragraphs 31-37.

42. A method of creating a wheat plant that is resistant to the herbicide glyphosate compared to wild type wheat plants, comprising the steps of:

- a. obtaining plant material from a parent wheat plant;
- b. inducing at least one mutation in at least one copy of an EPSPS gene of the plant material by treating the plant material with a mutagen to create mutagenized plant material;
- c. culturing the mutagenized plant material to produce progeny wheat plants;
- d. analyzing progeny wheat plants to detect at least one mutation in at least one copy of an EPSPS gene;
- e. selecting progeny wheat plants that are resistant to the herbicide glyphosate compared to the parent wheat plant; and
- f. repeating the cycle of culturing the progeny wheat plants to produce additional progeny wheat plants that are resistant to glyphosate.

43. The method of paragraph 42 wherein the plant material is selected from the group consisting of seeds, pollen, plant cells, or plant tissue.

44. The method of paragraph 42 wherein the mutagen is ethyl methanesulfonate.

45. The method of paragraph 44 wherein the concentration of ethyl methanesulfonate used is from 0.75% to about 1.2%.

46. The method of paragraph 42 where the progeny wheat plant are analyzed by

- a. isolating genomic DNA from the progeny wheat plants; and

b. amplifying segments of an EPSPS gene in the isolated genomic DNA using primers specific to the EPSPS gene or to the DNA sequences adjacent to the EPSPS gene.

47. The method of any of the preceding paragraphs wherein the EPSPS gene sequence is substantially identical to SEQ. I.D. No.: 6.

48. The method of any of the preceding paragraphs wherein the EPSPS gene sequence is substantially identical to SEQ. I.D. No.: 7.

49. The method of any of the preceding paragraphs wherein the EPSPS gene sequence is substantially identical to SEQ. I.D. No.: 8.

50. The method of any of the preceding paragraphs where at least one primer has a sequence substantially identical to a sequence in the group consisting of SEQ. ID. NOs. 9 through 12.

51. The method of any of the preceding paragraphs wherein the mutation detected in step d is evaluated to determine the mutation's likelihood of increasing resistance to glyphosate

52. The method of any of the preceding paragraphs where in the mutation is evaluated using a bioinformatics tool selected from the group consisting of SIFT, PSSM and PARSESNP.

53. A wheat plant created according to a method of any of the preceding paragraphs

54. Flower, seeds, pollen, plant parts or progeny of the wheat plant of paragraph 53.

55. Parts of the seeds of paragraph 54.

56. Food and food products incorporating any portion of the seed of the wheat plant of paragraph 53.

57. A wheat plant exhibiting resistance to glyphosate created by breeding a wheat plant with the wheat plant of paragraph 53.

58. An endogenous EPSPS gene having substantial identity to SEQ. I.D. No. 6 and having a non-transgenic mutation within the endogenous EPSPS gene following treatment with a mutagen.

59. The endogenous EPSPS gene of paragraph 58 wherein the non-transgenic mutation is a missense mutation.

60. The non-transgenic missense mutation of paragraph 59 wherein the mutation is a T168I of SEQ ID NO. 9.

61. The non-transgenic missense mutation of paragraph 59 wherein the mutation is a P172S of SEQ ID NO. 9.

62. A wheat plant containing the endogenous EPSPS gene of any of the preceding paragraphs.

63. Flowers, seeds, pollen, plant parts, and progeny of the wheat plant of any of the preceding paragraphs.

64. Parts of the seeds of any of the preceding paragraphs.

65. Food and food products incorporating any portion of the seeds of the wheat plant of any of the preceding paragraphs.

66. An endogenous EPSPS gene having substantial identity to SEQ. I.D. No. 7 and having a non-transgenic mutation within the endogenous EPSPS gene following treatment with a mutagen.

67. The endogenous EPSPS gene of paragraph 66 wherein the non-transgenic mutation is a missense mutation.

68. The non-transgenic missense mutation of paragraph 67 wherein the mutation is a T168I of SEQ ID NO. 9.

69. The non-transgenic missense mutation of paragraph 67 wherein the mutation is a P172S of SEQ ID NO. 9.

70. A wheat plant containing the endogenous EPSPS gene of paragraph 66.

71. Flowers, seeds, pollen, plant parts, and progeny of the EPSPS plant of any of the preceding paragraphs.

72. Parts of the seeds of any of the preceding paragraphs.

73. Food and food products incorporating any portion of the seeds of the wheat plant of any of the preceding paragraphs.

74. An endogenous EPSPS gene having substantial identity to SEQ. I.D. No. 8 and having a non-transgenic mutation within the endogenous EPSPS gene following treatment with a mutagen.

75. The endogenous EPSPS gene of paragraph 74 wherein the non-transgenic mutation is a missense mutation.

76. The non-transgenic missense mutation of paragraph 75 wherein the mutation is a threonine to isoleucine at position 173 (T173I) of SEQ ID NO. 3 or T168I of SEQ ID NO. 9.

77. The non-transgenic missense mutation of claim 75 wherein the mutation is a proline to serine at position 177 of SEQ ID NO. 3 (P177S) or P172S of SEQ ID NO. 9.

78. A wheat plant containing the endogenous EPSPS gene of paragraph 74.

79. Flowers, seeds, pollen, plant parts, and progeny of the EPSPS plant of any of the preceding paragraphs.

80. Parts of the seeds of any of the preceding paragraphs.

81. Food and food products incorporating any portion of the seeds of the wheat plant of any of the preceding paragraphs.

82. A wheat plant containing two or more of the endogenous EPSPS genes selected from the endogenous EPSPS genes of any of the preceding paragraphs.

83. A wheat plant comprising a mutation in an EPSPS gene of at least two of the A, B, and D genomes, wherein the EPSPS gene of the A genome encodes an EPSPS polypeptide comprising a proline to serine mutation at amino acid position 172 of SEQ ID NO. 9 (P177S of SEQ ID NO. 3).

84. A wheat plant comprising at least two mutations in an EPSPS gene of the A genome, wherein the mutated EPSPS gene of the A genome encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9 (T173I and P177S of SEQ ID NO. 3).

85. A wheat plant comprising at least two mutations in an EPSPS gene of the D genome, wherein the mutated EPSPS gene of the D genome encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9 (T173I and P177S of SEQ ID NO. 3).

86. A wheat plant comprising a mutation in an EPSPS gene of at least two of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9 (T173I and P177S of SEQ ID NO. 3).

87. A wheat plant comprising a mutation in an EPSPS gene of at least two of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9.

88. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the B genome has a mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 of SEQ ID NO. 9.

89. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome has at least one mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and/or a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the B genome has a mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 of SEQ ID NO. 9.

90. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least one mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and/or a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the B genome has a mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 of SEQ ID NO. 9.

91. A wheat plant comprising a mutation in an EPSPS gene of at least two of the A, B, and D genomes, wherein the EPSPS gene of the A genome encodes an EPSPS polypeptide comprising a proline to serine at amino acid position 172 of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome encodes an EPSPS polypeptide comprising a threonine to isoleucine at amino acid position 168 of SEQ ID NO. 9.

92. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least two mutations, the EPSPS gene of the B genome has a least one mutation, and the EPSPS gene of the D genome has at least one mutation, wherein the wheat plant has increased resistance to glyphosate.

93. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least one mutations, the EPSPS gene of the B genome has a least one mutation, and the EPSPS gene of the D genome has at least two mutations, wherein the wheat plant has increased resistance to glyphosate.

94. A wheat plant comprising a mutation in an EPSPS gene in each of the A, B, and D genomes, wherein the EPSPS gene of the A genome has at least one mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and/or a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome has at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the B genome has a mutation and encodes an EPSPS polypeptide comprising a proline to leucine change at amino acid position 172 of SEQ ID NO. 9.

95. The wheat plant of any of paragraphs 82-94, which is *Triticum aestivum* ssp. *aestivum*.

96. The wheat plant of any of paragraphs 82-94, which is *Triticum turgidum* subsp. *Durum*.

97. Wheat grain from the wheat plant of any of paragraphs 82-94.

98. Flour comprising the wheat grain of paragraph 97.

99. A food product comprising a component of the wheat plant of paragraphs 82-94

100. A wheat seed, plant part or progeny thereof from the wheat plant of paragraphs 82-94.

101. A wheat plant of any of the preceding paragraphs, wherein the wheat plant has resistance to glyphosate.

102. A wheat plant of any of the preceding paragraphs, wherein the wheat plant has resistance to glyphosate and unaltered growth characteristics.

103. A wheat plant of any of the preceding paragraphs, wherein the EPSPS protein has reduced affinity for glyphosate and substantial affinity for plant or endogenous substrates.

The following Examples are offered by way of illustration only, and not limitation. It is to be understood that the mutations discussed herein are merely exemplary and that similar mutations are also contemplated.

EXAMPLES

Example 1

A. Mutagenesis

Wheat seeds of the hexaploid cultivar Express (*Triticum aestivum*, PVP #9000012) were vacuum infiltrated in H₂O (approximately 1,000 seeds/100 ml H₂O for approximately 4 minutes). The seeds were then placed on a shaker (45 rpm) in a fume hood at ambient temperature. The mutagen ethyl methanesulfonate (EMS) was added to the imbibing seeds to final concentrations ranging from about 0.75% to about 1.2% (v/v). Following an 18 hour incubation period, the EMS solution was replaced 4 times with fresh H₂O. The seeds were then rinsed under running water for about 4-8 hours. Finally, the mutagenized seeds were planted (96/tray) in potting soil and allowed to germinate indoors. Plants that were four to six weeks old were transferred to the field to grow to fully mature M1 plants. The mature M1 plants were allowed to self-pollinate and then seeds from the M1 plant were collected and planted to produce M2 plants.

B. DNA Preparation

DNA from these M2 plants produced in accordance with the above description was extracted and prepared in order to identify the M2 plants carried a mutation at an EPSPS locus. The M2 plant DNA was prepared using the methods and reagents contained in the Qiagen® (Valencia, Calif.)

DNeasy® 96 Plant Kit. Approximately 50 mg of frozen plant sample was placed in a sample tube with a tungsten bead, frozen in liquid nitrogen and ground 2 times for 1 minute each at 20 Hz using the Retsch® Mixer Mill MINI 300. Next, 400 µl of solution AP1 [Buffer AP1, solution DX and RNase (100 mg/ml)] at 80° C. was added to the sample. The tube was sealed and shaken for 15 seconds. Following the addition of 130 µl Buffer AP2, the tube was shaken for 15 seconds. The samples were placed in a freezer at minus 20° C. for at least 1 hour. The samples were then centrifuged for 20 minutes at 5,600×g. A 400 µl aliquot of supernatant was transferred to another sample tube. Following the addition of 600 µl of Buffer AP3/E, this sample tube was capped and shaken for 15 seconds. A filter plate was placed on a square well block and 1 ml of the sample solution was applied to each well and the plate was sealed. The plate and block were centrifuged for 4 minutes at 5,600×g. Next, 800 µl of Buffer AW was added to each well of the filter plate. Plates were sealed and spun for 15 minutes at 5600×g in the square well block. The filter plate was then placed on a new set of sample tubes and 80 µl of Buffer AE was applied to the filter. It was capped and incubated at room temperature for 1 minute and then spun for 2 minutes at 5,600×g. This step was repeated with an additional 80 µl Buffer AE. The filter plate was removed and the tubes containing the pooled filtrates were capped. The individual samples were then normalized to a DNA concentration of 5 to 10 ng/µl.

C. Tilling®

The M2 DNA was pooled into groups of two individual plants. The DNA concentration for each individual within the pool was approximately 0.8 ng/µl with a final concentration of 1.6 ng./µl for the entire pool. Then, 5 µl of the pooled DNA samples (or 8 ng) was arrayed on microtiter plates and subjected to gene-specific PCR.

PCR amplification was performed in 15 µl volumes containing 2.5 ng pooled DNA, 0.75×ExTaq buffer (Panvera®, Madison, Wis.), 2.6 mM MgCl₂, 0.3 mM dNTPs, 0.3 µM primers, and 0.05 U Ex-Taq (Panvera®) DNA polymerase. PCR amplification was performed using an MJ Research® thermal cycler as follows: 95° C. for 2 minutes; 8 cycles of “touchdown PCR” (94° C. for 20 second, followed by annealing step starting at 70-68° C. for 30 seconds and decreasing 1° C. per cycle, then a temperature ramp of 0.5° C. per second to 72° C. followed by 72° C. for 1 minute); 25-45 cycles of 94° C. for 20 seconds, 63-61° C. for 30 seconds, ramp 0.5° C./sec to 72° C., 72° C. for 1 minute; 72° C. for 8 minutes; 98° C. for 8 minutes; 80° C. for 20 seconds; 60 cycles of 80° C. for 7 seconds-0.3 degrees/cycle.

The PCR primers (MWG Biotech, Inc., High Point, N.C.) were mixed as follows:

- 2.5 µl 100 µM IRD-700 labeled left primer
- 7.5 µl 100 µM left primer
- 9.0 µl 100 µM IRD-800 labeled right primer
- 1.0 µl 100 µM right primer

A label can be attached to each primer as described or to only one of the primers. Alternatively, Cy5.5 modified primers could be used. The label was coupled to the oligonucleotide using conventional phosphoramidite chemistry.

PCR products (15 µl) were digested in 96-well plates. Next, 30 µl of a solution containing 10 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (pH 7.5), 10 mM MgSO₄, 0.002% (w/v) Triton X-100, 20 ng/ml of bovine serum albumin, and CEL 1 (Transgenomic®, Inc.; 1:100,000 dilution) was added with mixing on ice, and the plate was incubated at 45° C. for 15 minutes. The specific activity of the CEL1 was 800 units/µl, where a unit was

defined by the manufacturer as the amount of enzyme required to produce 1 ng of acid-soluble material from sheared, heat denatured calf thymus DNA at pH 8.5 in one minute at 37° C. Reactions were stopped by addition of 10 µl of a 2.5 M NaCl solution with 0.5 mg/ml blue dextran and 75 mM EDTA, followed by the addition of 80 µl isopropanol. The reactions were precipitated at 80° C., spun at 4,000 rpm for 30 minutes in an Eppendorf Centrifuge 5810. Pellets were resuspended in 8 µl of 33% formamide with 0.017% bromophenol blue dye, heated at 80° C. for 7 minutes and then at 95° C. for 2 minutes. Samples were transferred to a membrane comb using a comb-loading robot (MWG Biotech). The comb was inserted into a slab acrylamide gel (6.5%), electrophoresed for 10 minutes, and removed. Electrophoresis was continued for 4 hours at 1,500-V, 40-W, and 40-mA limits at 50° C.

During electrophoresis, the gel was imaged using a LICOR® (Lincoln, Nebr.) scanner which was set at a channel capable of detecting the IR Dye 700 and 800 labels. The gel image showed sequence-specific pattern of background bands common to all 96 lanes. Rare events, such as mutations, create new bands that stand out above the background pattern. Plants with bands indicative of mutations of interest were evaluated by TILLING® individual members of a pool mixed with wild type DNA and then sequencing individual PCR products. Plants carrying mutations confirmed by sequencing were grown up as described above (e.g., the M2 plant was backcrossed or outcrossed twice in order to eliminate background mutations and self-pollinated in order to create a plant that was homozygous for the mutation).

D. Identification and Evaluation of Mutation P177S in A Genome

DNA from a wheat plant originating from seeds of cultivar Express, which were incubated in 0.75% EMS, was amplified using primers TaEPS1CL and Ep486AR (SEQ ID NOs: 10 and 11). The PCR amplification products were then incubated with CEL 1 and electrophoresed. The electrophoresis gel image showed an IRD-700 labeled fragment at approximately 200 bp, which stood out above the background pattern for the full length PCR amplification product that was approximately 486 bp. Therefore, it was likely that this fragment contained a heteroduplex created by a mutation in the A homoeologue of the EPSPS gene sequence. Sequence analysis of this fragment showed the mutation was a cytosine to thymine change at nucleotide 1233 numbered according to the published genomic DNA for rice EPSPS (SEQ ID NO: 2). This mutation was associated with a change from proline to serine at amino acid number 177 (numbered according to the published rice EPSPS protein SEQ ID NO: 3) in the EPSPS polypeptide coded for by the A genome EPSPS homoeologue.

E. Identification and Evaluation of Mutation T173I in the B Genome

DNA from a wheat plant originating from seeds of cultivar Express that were incubated in 1.2% EMS, was amplified using primers TaEPS1CL and Ep558BR (SEQ ID NOs: 10 and 12). The PCR amplification products were then screened using a modification of the TaqMan® SNP Genotyping Assay (PN4332856C available from Applied Biosystems, Foster City, Calif.) with PCR primers (SEQ ID NOs: 14-15) and TaqMan® MGB probes (SEQ ID NOs: 16-17) to identify a cytosine to thymine change at nucleotide 1222 numbered according to the published genomic DNA for rice EPSPS (SEQ ID NO: 2). This mutation was associated with a change from threonine to isoleucine at amino acid number 173 (numbered according to the published rice EPSPS

protein SEQ ID NO: 3) in the EPSPS polypeptide coded for by the B genome EPSPS homoeologue.

F. Identification and Evaluation of Mutation T173I in the D Genome

DNA from a wheat plant originating from seeds of cultivar Express that were incubated in 1.0% EMS, was amplified using primers TaEPS1CL and TaEPSJR (SEQ ID NOs: 10 and 13). The PCR amplification product were then incubated with CEL 1 and electrophoresed. The electrophoresis gel image showed an IRD_700 labeled fragment approximately 160 bp in length, which stood out above the background pattern for the full length PCR amplification product which was approximately 1,000 bps in length. Therefore, it was likely that this fragment contained a heteroduplex created by a mutation in the B homoeologue of the EPSPS gene sequence. Sequence analysis of this fragment showed the mutation was a cytosine to thymine change at nucleotide 1222 numbered according to the published genomic DNA for rice EPSPS (SEQ ID NO: 2). This mutation was associated with a change from threonine to isoleucine at amino acid number 173 (numbered according to the published rice EPSPS protein SEQ ID NO: 3) in the EPSPS polypeptide coded for by the D genome EPSPS homoeologue.

G. Phenotypic Analysis:

Wheat Plants Selected for Study:

Plants homozygous for the single preferred mutations (the T173I mutation in the B genome, the T173I mutation in the D genome and the P177S mutation in the A genome) were identified in the M3 seeds. Wild type siblings were also identified and used as controls. Plants that were homozygous for the T173I mutation in the D genome were crossed to plants that were homozygous for the P177S mutation in the A genome to generate double mutant plants.

Measurement of Resistance to Glyphosate:

Growth Chamber Studies: Seeds from plants that were homozygous for one of the single preferred mutations and seeds from their wild type sibling control plants were surface sterilized, placed on moist germination paper overnight, and then grown in glass tubes with 0.1× Murashige & Skoog (MS) medium containing 0 or 0.15 mM glyphosate. Germination was performed in a growth chamber under a light condition of 16 hours of light/8 hours of dark of 80 microEinsteins. At 10-14 days of development, shoot and root lengths were measured. On medium containing glyphosate, both shoot and root length were significantly greater for seeds that were homozygous for the preferred mutations than for wild type sibling control seeds indicating that the seeds with preferred mutations were resistant to glyphosate. Wild type seeds failed to thrive on medium containing glyphosate and most failed to grow whereas the majority of seeds with mutations grew well.

Seeds from plants that were homozygous for the double preferred mutations (both T173 I and P177S) were compared to seeds that were homozygous for each of the single preferred mutation for their ability to survive on MS media containing glyphosate. Seeds from their wild type sibling plants were used as positive controls. Seeds were germinated as described above. Measurements of shoot and root length indicated that seeds homozygous for the double preferred mutations were more resistant to glyphosate than seeds that were homozygous for each of the single preferred mutations. All three groups of seeds carrying mutations in one or more EPSPS genes (double T173I and P177S; single T173I; single P177S) were resistant to glyphosate and grew well compared to seeds from wild type sibling plants which for the most part failed to grow.

H. Re-Mutagenesis of Seeds of the Plant Homozygous for A: P177S and D: 173I

In one embodiment, re-mutagenesis of seeds of plants homozygous for a mutation in the A genome or D genome can identify second-site mutations. Progeny seeds of the single site A and D genome plant were re-mutagenized and independent M2 mutants were identified that contained second-site mutations in EPSPS. A plant was identified that contains both the mutations P177S and T173I in the A genome. This combination was identified two independent times. Separately a plant was identified that contains the mutations P177S and T173I in the D genome. Crosses were made to incorporate both double mutants in the A and D genomes into a single plant. Separately, the B genome T173I mutation has been crossed to a plant containing the double mutations in the A and D genomes. In addition, separately, the B genome P177L (P172L) was crossed to a plant containing the double mutations in the A and D genomes.

The above examples are provided to illustrate the disclosure but not limit its scope. Other variants of the disclosure

will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims and all their equivalents. The examples above used TILLING technology to create and identify mutations in one or more EPSPS genes of wheat but one of ordinary skill in the art would understand that other methods such as targeted mutagenesis (also known as site-directed mutagenesis, site-specific mutagenesis or oligonucleotide-directed mutagenesis) could be used to create the useful mutations in one or more EPSPS loci of wheat (see for example Zhang et al., *PNAS* 107(26):12028-12033, 2010; Saika et al., *Plant Physiology* 156:1269-1277, 2011). One of ordinary skill in the art would also recognize that additional methods could be used to inactivate or reduce the activity of the wheat EPSPS genes. These methods include without limitation CRISPR/Cas9 mutagenesis, TALEN and zinc finger mutagenesis, RNAi, micro RNA and hairpin RNA based methods to mutate or reduce the accumulation of the EPSPS transcripts. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

INFORMATIONAL SEQUENCE LISTING:

SEQ ID NO: 1 shows the *Oryza sativa* EPSPS mRNA (NCBI Reference Sequence NM_001063247).
ORIGIN

```

1   atggcggcga ccatggcgtc caacgccgcg gctgcggcgg cgggtgccct ggaccaggcc
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181 gcgctcgtcg cgtcgggtgc agcgcggcg gcgaaggcgg aggagatcgt gctccagccc
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INFORMATIONAL SEQUENCE LISTING:

1561 aaagagtgag gtctaggttc tgttg

SEQ ID NO: 2 shows the *Oryza sativa* EPSPS genomic DNA (NCBI Reference Sequence NC_008399).

ORIGIN

1 atggcggcga ccatggcgtc caacgccgcg gctgcggcgg cgggtgccct ggaccaggcc
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 301 ctctctctct cgcctctctc cgaggtaga gcgggatccc ttctcttgc gtgaattcca
 361 tttctggaga tgagatttta gggggtttat taggtgaggt ggctgtgttt gtgaaatcct
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 481 cacactcata taaccaattt attgaaacat tttggtttgg cataagaaac tgcttacgaa
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 661 tttggaggtt tgcactgta ccaatgttat gtttgaacat tttgcaagca gtgctttctc
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INFORMATIONAL SEQUENCE LISTING:

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 2401 tccctgacct acatgttaat attacagggt gatgtcaaat ttgctgaggt acttgagatg
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 3301 gttg

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SEQ ID NO: 3 shows the EPSPS protein encoded by SEQ ID NO: 2 (NCBI Reference Sequence NP_001056712).

ORIGIN

1 maatmasnaa aaaaysldqa vaasaafssr kqlrlpaaar ggmrvrvrar grreavvvas
 61 assssvaapa akaeeivlqp ireisgavql pgskslsnri lllsalsegt tvvdnllnse
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 181 avtaaggnat yvldgvprmr erpigdlvvg lkqlgadvc flgtecppvr vkgigglpgg
 241 kvklsgsiss qylsallmaa plalgdveie iidklisipy vemtlrlmer fgvkaehsds
 301 wdrfyikggq kykspgnayv egdassasyf lagaaitggt vtvqgcgts lqgdvkfaev
 361 lemmgakvtw tdtsvtvtgp prepygkhl kavdvnmnkm pdvamtlavv alfadgptai
 421 rdvaswrvke termvairte ltklgasvee gpdyciitpp eklnitaidt yddhrmamaf
 481 slaacadvpv tirdpgctrk tfpnyfdvls tfvrn

SEQ ID NOs: 4-5 show the DNA sequences for *Triticum aestivum* EPSPS-specific primers of used for genomic sequencing.

SEQ ID	Primer Name	Primer ID	Primer Sequence
4	TaEPS1CL	3155	ACAGTGAGGATGTCCACTACATGCTTGA
5	TaEPS1ER	3158	AAATAGCTCGCACTTGAGGCATCACCTT

SEQ ID NO: 6 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the A genome of wheat.

GACCTTGGATCTCCGTGgAGCAGATAAAGTTGCAAAAAGAGCTGTGGTTGTTGGCTGTGGCGGCAGGTTCCAGTCGAA
 AAGGACGCCAAAGAGGAAGTAAAGCTCTTCTTGGGTAATGCTGGAAGTCAATGCGGCCACTGACGGCAGCTGTAGTAGC

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INFORMATIONAL SEQUENCE LISTING:

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CATACTCCTCTTATGTACTCGGAAACTGTTAGTCCAACTTCATAAAAGTTGCAGTAAC

SEQ ID NO: 7 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the B genome of wheat.

CCTTGGATCTCCGTGGAAGCAGATAAAGTTGCAAAAAGAGCTGTGGTTGTTGGCTGTGGCGGAGGTTCCCGGTCGAAAA
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GGAATCGACAG

SEQ ID NO: 8 shows the DNA sequence of a PCR product that comprises a partial genomic DNA sequence for EPSPS for the D genome of wheat

ACGCCAAAGAGGAAGTAAAGCTCTTCTTGGGTAATGCTGGAAGTCAATGCGTCCATTGACGGCAGCTGTAGTAGCTGCT
GGTGGAAATGCAACGTATGTTTTCTTTATCCTAGTTGAAATAAGTATGAGATCCATGGGTATGTTTGGAGACTGATCGTG
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GCTTCTTTTGGCCCTTTTATCTTAGGCCCTTGCTGCCATATGGCCCATCAGGATACACATCCATGCTTTTACTGAACAT
ACTCTTATGTACTCGGAAACTGTTAGTTTCAACTTCATAAAAGTTGCAGTAACCATTTCTTAAACGAGCCATCCGTTCTC

SEQ ID NO: 9 shows the amino acid sequence of the wheat EPSPS protein.

MAMAAATVAASAS SVAVSLDRAAPAHPRRLRMPAARAHRGAVRLWGPRGAARATSVAAAPAPAGAE
VVLQPIREISGAVQLPGSKSLSNRILLLSALSSEGTTVVDNLLNSEDVHYM
LEALEALGLSVEADKVAKRAVVVCGGRFPVEKDAKEEVKLFGLGNAGTAM
RPLTAAVVAAGGNATYVLDGVPRMRERPIGDLVGLQQLGADVDCFLGTN
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SASYELAGAAITGGTVTVEGCGTSLQGDVKFAEVLEMMGAKVTWTDTSV
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WRVKETERMVAIRTELTKLGATVEEGPDYCIITPPEKLNITAIIDTYDDHR
MAMAFSLAACAEVPTIRDPGCTRKTFPNYEDVLSFVKN*

SEQ ID NOs: 10-13 show the DNA sequences for wheat EPSPS-specific primers used for mutation detection.

SE ID	Primer Name	Primer ID	Primer Sequence
10	TaEP51CL	3155	ACAGTGAGGATGTCCACTACATGCTTGA
11	Ep486AR	3418	ACTTCTCTGACAGAGAACAGAAGTGTGCAC

- continued

INFORMATIONAL SEQUENCE LISTING:

12	Ep558BR	3419	TTGTGTAAGGTCGCATTGATCGTACTACCA
13	TaEPSJR	3223	GAAACTAGAAATCATGCTTTTGTACTCCACTATC

SEQ ID NOS: 14-17 show TaqMan[®] Primers Useful for T173I Mutation Detection.

SEQ ID	Primer Name	Primer Sequence
14	HT TtoI-TtoIF	ACGCCAAAGAGGAAGTAAAGCT
15	HT TtoI-TtoIR	TCCAAACATACCCATGGATCTCATACT
16	HT TtoI-TtoIV2 VIC	CGCATTGCAGTTCCA
17	HT TtoI-TtoIM2 FAM	CATTGCAATTCCA

SEQ ID NO. 18 shows the active region of the wheat EPSPS protein:
FLGNAGTAMRPLTAAVVAAGGN.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 21

<210> SEQ ID NO 1

<211> LENGTH: 1585

<212> TYPE: DNA

<213> ORGANISM: *Oryza sativa*

<400> SEQUENCE: 1

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tgggacagat tctatattaa gggagggcag aagtacaaat ctctggaaa tgccatggt      960
gaaggtgatg cctcaagcgc gagctatttc ttggctggtg ctgcaatcac tggaggcact     1020
gtgacagttc aaggttgtg tacgaccagt ttgcagggtg atgtcaaatt tgctgaggta     1080
ctgagatga tgggagcaaa ggttacatgg actgacacca gtgtaaccgt aactggtcca     1140
ccacgtgagc cttatgggaa gaaacacctg aaagctgttg atgtcaacat gaacaaaatg     1200
cctgatgttg ccatgacct tgccgttgtt gcactcttcg ctgatggtcc aactgctatc     1260

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agagatgtgg cttcctggag agtaaaggaa accgaaagga tggttgcaat tcggaccgag 1320
ctaacaaagc tgggagcadc ggttgaagaa ggtcctgact actgcatcat caccaccacg 1380
gagaagctga acatcacggc aatcgacacc tacgatgac acaggatggc catggccttc 1440
tcctcgcctg cctgcgccga cgtgcccgctg acgatcaggg accctgggtg cacccgcaag 1500
accttcccc aactacttca cgttctaagc actttcgtca ggaactgaac tgagctttta 1560
aaagagtggg gtctaggttc tgttg 1585

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<210> SEQ ID NO 2
<211> LENGTH: 3304
<212> TYPE: DNA
<213> ORGANISM: Oryza sativa

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<400> SEQUENCE: 2

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atggcggcga ccatggcgtc caacgccgctg gctgcggcgg cgggtgtccct ggaccaggcc 60
gtggcggcgt cggcggcgtt ctcgtcgcgg aagcagctgc ggctgcccgc cgcggcgcgc 120
ggggggatgc ggggtgcgggt gcgggcgcgg gggcggcggg aggcgggtgt ggtggcgtcc 180
gcgtcgtcgt cgtcgggtgc agcgcggcgc gcgaaggcgg aggagatcgt gctccagccc 240
atcagggaga tctccggggc ggttcagctg ccagggtcca agtcgctctc caacaggatc 300
ctcctcctct ccgccctctc cgagggtgaga cgcggatccc ttcctcttgc gtgaattcca 360
tttctggaga tgagatttta gggggtttat taggtgaggt ggctgtgttt gtgaaatcct 420
aggaattatc tctcaagtca atctaacgat gagatataac tgaggttctg gttttaatca 480
cactcacta taaccaattt attgaaacat tttggtttgg cataagaaac tgcttacgaa 540
ggtatgatat cctcctacat gtcaggctac taaattttca cgacgggatg atccactcaa 600
aacaagtffc ttaacgagtc tggtgaggtc tgttatgaaa tttgtgtaa ctaaggcaac 660
tttgagggtt tcgcactgta ccaatgttat gtttgaacat tttgcaagca gtgctttctc 720
ccaaaattat gcaattttga ggctcctcta catcattata attccccaat acattgctct 780
ttattcttaa tagctttgat cgcgaaattt aacattttta ttcttgagct gttattttgt 840
agcatcagtt tatcatgagc catgtttggt actaaatata caatcccttg ggtttatttg 900
ttccaagca tgcattaac ttatcttaat gtggacaaga aactgatgcc tgcttacatt 960
gctattattt caagcgggta ttgatccttt gacatgtgat tgatcatttt tttttctctg 1020
gttattaggg cacaacagtg gtggacaact tgctgaacag tgaggatgtt cactacatgc 1080
ttgagccct gaaagccctc gggctctctg tggaaagcaga taaagttgca aaaagagctg 1140
tagtcgttgg ctgtggtggc aagtttcctg ttgagaagga tgcgaaagag gaagtgcac 1200
tcttcttggg gaacgctgga actgcaatgc gaccattgac agcagccgtg actgctgctg 1260
gtggaaatgc aacgtatgtt tttttttta atgtttatga aaatattgat ggaattcatg 1320
gggtatgttt tatgacctt ttctttacca tcagttatgt gcttgatgga gtgccacgaa 1380
tgagggagag accgattggt gacttggttg tcgggttgaa acaacttggg gcggatgtcg 1440
actgtttcct tggcactgaa tgcccacctg ttcgtgtcaa ggaattgga ggacttctctg 1500
gtggcaagggt tagttactcc taaactgcat cctttgtact tctgtatgca cctcaattct 1560
ttgtcaacct tctgcattta taaggaacat tctatgatgc aattcgacct tacactgcac 1620
agtaacttga aatgtttcat gcttaataca tatgccatat tctgccaag ctcaagcgag 1680
caatatttgg ttgaatttgg taccatattt ttgtatattt gggcattcct ttttggctt 1740

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gatgtcttct tttgaattag catttaactg aattacactc aacagggttaa gctctctggt 1800
tccatcagca gtcagtactt gagtgccttg ctgatggctg ctcccttggc ccttggggat 1860
gtggagatcg aatcattga caaactaatc tccattcctt acgttgaaat gacattgaga 1920
ttgatggagc gttttggtgt gaaggcagag cattctgata gttgggacag attctatatt 1980
aagggagggc agaagtacaa gtaagcttct acctgcctta ctgagctgaa ttattcgggt 2040
gtttatgatt aactccctaa actaaccctt tttcttttct ttggcattga cagatctcct 2100
ggaaaatgct atgttgaagg tgatgcctca agcgcgagct atttcttggc tgggtgctgca 2160
atcactggag gcactgtgac agttcaaggt tgtggtacga ccagtttgca ggtataactg 2220
tagtgctgt tttgacattc taccgtttag tcaagtttag tcagtagtca catattcaga 2280
atatagcaca atctgtatta tgccactggt aatcaaatac gcttgacctg gagagtgcta 2340
tataccctag cttaatcttc aaactaaaca gttctcttgt ggcttgctgt gctgttatgt 2400
tcctgacct acatgttaat attacagggt gatgtcaaat ttgctgaggt acttgagatg 2460
atgggagcaa aggttacatg gactgacacc agtgaaccg taactggtcc accacgtgag 2520
ccttatggga agaaacacct gaaagctggt gatgtcaaca tgaacaaaat gcctgatggt 2580
gccatgacct ttgccgttgt tgcactcttc gctgatggtc caactgctat cagagatggt 2640
aaacattaag gcctattata cctgttctat catactagca attactgctt agcattgtga 2700
caaaacaaat aaccaaactt tcttcaaat aacttagaaa tataagaaag gttcgttttg 2760
tgtggtaaac agtactactg tagtttcagc tatgaagttt gctgctggca attttctgaa 2820
cgttttcagc taaattgcat gtttgttcat catacttacc cattgtcttc cacagtggct 2880
tcttgagag taaaggaaac cgaaaggatg gttgcaatc ggaccgagct aacaaaggta 2940
aattcattag gtccctgtc ctttcattct tcaagtagtt tgttcataag ttgaattctc 3000
cttcaatgat gtttaaattc atcatcttct tttttggtgt tgtgccagct gggagcatcg 3060
gttgaagaag gtctgacta ctgcatcatc accccaccgg agaagctgaa catcacggca 3120
atcgacacct acgatgatca caggatggcc atggccttct ccctcgctgc ctgcgccgac 3180
gtgcccgtga cgatcaggga ccttggttgc acccgcaaga ccttcccca ctacttcgac 3240
gttctaagca ctttcgctcag gaactgaact gagcttttaa aagagtgagg tctaggttct 3300
gttg 3304

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<210> SEQ ID NO 3
<211> LENGTH: 515
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

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<400> SEQUENCE: 3

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Met Ala Ala Thr Met Ala Ser Asn Ala Ala Ala Ala Ala Val Ser
1           5           10           15
Leu Asp Gln Ala Val Ala Ala Ser Ala Ala Phe Ser Ser Arg Lys Gln
           20           25           30
Leu Arg Leu Pro Ala Ala Ala Arg Gly Gly Met Arg Val Arg Val Arg
           35           40           45
Ala Arg Gly Arg Arg Glu Ala Val Val Val Ala Ser Ala Ser Ser Ser
           50           55           60
Ser Val Ala Ala Pro Ala Ala Lys Ala Glu Glu Ile Val Leu Gln Pro
65           70           75           80
Ile Arg Glu Ile Ser Gly Ala Val Gln Leu Pro Gly Ser Lys Ser Leu
           85           90           95

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Ser Asn Arg Ile Leu Leu Leu Ser Ala Leu Ser Glu Gly Thr Thr Val
 100 105 110
 Val Asp Asn Leu Leu Asn Ser Glu Asp Val His Tyr Met Leu Glu Ala
 115 120 125
 Leu Lys Ala Leu Gly Leu Ser Val Glu Ala Asp Lys Val Ala Lys Arg
 130 135 140
 Ala Val Val Val Gly Cys Gly Gly Lys Phe Pro Val Glu Lys Asp Ala
 145 150 155 160
 Lys Glu Glu Val Gln Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg
 165 170 175
 Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly Asn Ala Thr Tyr Val
 180 185 190
 Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val
 195 200 205
 Val Gly Leu Lys Gln Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr
 210 215 220
 Glu Cys Pro Pro Val Arg Val Lys Gly Ile Gly Gly Leu Pro Gly Gly
 225 230 235 240
 Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ala Leu
 245 250 255
 Leu Met Ala Ala Pro Leu Ala Leu Gly Asp Val Glu Ile Glu Ile Ile
 260 265 270
 Asp Lys Leu Ile Ser Ile Pro Tyr Val Glu Met Thr Leu Arg Leu Met
 275 280 285
 Glu Arg Phe Gly Val Lys Ala Glu His Ser Asp Ser Trp Asp Arg Phe
 290 295 300
 Tyr Ile Lys Gly Gly Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr Val
 305 310 315 320
 Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile
 325 330 335
 Thr Gly Gly Thr Val Thr Val Gln Gly Cys Gly Thr Thr Ser Leu Gln
 340 345 350
 Gly Asp Val Lys Phe Ala Glu Val Leu Glu Met Met Gly Ala Lys Val
 355 360 365
 Thr Trp Thr Asp Thr Ser Val Thr Val Thr Gly Pro Pro Arg Glu Pro
 370 375 380
 Tyr Gly Lys Lys His Leu Lys Ala Val Asp Val Asn Met Asn Lys Met
 385 390 395 400
 Pro Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly
 405 410 415
 Pro Thr Ala Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu
 420 425 430
 Arg Met Val Ala Ile Arg Thr Glu Leu Thr Lys Leu Gly Ala Ser Val
 435 440 445
 Glu Glu Gly Pro Asp Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn
 450 455 460
 Ile Thr Ala Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe
 465 470 475 480
 Ser Leu Ala Ala Cys Ala Asp Val Pro Val Thr Ile Arg Asp Pro Gly
 485 490 495
 Cys Thr Arg Lys Thr Phe Pro Asn Tyr Phe Asp Val Leu Ser Thr Phe
 500 505 510

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 Val Arg Asn
 515

<210> SEQ ID NO 4
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

<400> SEQUENCE: 4

acagtgagga tgtccactac atgcttga 28

<210> SEQ ID NO 5
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

<400> SEQUENCE: 5

aaatagctcg cacttgaggc atcacctt 28

<210> SEQ ID NO 6
 <211> LENGTH: 1339
 <212> TYPE: DNA
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 6

gaccttgat ctccgtggaa gcagataaag ttgcaaaaag agctgtgggt gttggctgtg 60
 gcggcagggt cccagtcgaa aaggacgcca aagaggaagt aaagctcttc ttgggtaatg 120
 ctggaactgc aatgcggcca ctgacggcag ctgtagtagc tgctgggtgga aatgcaacgt 180
 atgttttctt taccctagt gaaataagta tgagatccat gggatggtt ggagactgat 240
 cgtttctttt attaaaaaaa aacttcagtt atgtgcttga tggcgtacca agaatgaggg 300
 agcgacctat tggtagctta gttgtagggt tgcaacaact cggcgcagat gtcgattggt 360
 tccttgacc aaactgcca cctgtccgta tcaacggcaa aggaggtcta cctgggtggca 420
 aggttagcta ctcatcaact tgcattggtt ctactttgtg cacacttctg ttctctgtca 480
 gagaagttat tattatggct agtacgatgt aatgcaacct tagaccgccc actaaatttt 540
 aagaaccaat caacatttaa taatttcaat atgtgagtat tgcattgatc atgatagctt 600
 ggtgctaaag attgaatgat ttcacagggt aagctctctg gttccattag cagtcaatac 660
 ctgagttcct tgctgatggc tgctcctttg gctcttgagg atgtcgagat tgaaatcatt 720
 gataaactga tctccgttcc ttatggtgaa atgacattga aattgatgga gcgttttggg 780
 gtgactgccc agcattctga tagttgggac agattctaca ttaagggagg acaaaaatac 840
 aagcaagttc tacattttcc tacttcttct aataaacttt taattgtttc atttcttcta 900
 atagtggagt actaatgaaa gatatcagtt ttcagtttgc caaatattta cagacgcaac 960
 cccattgaat tatgggtgaa ctaggaaata aatgttttac taaaactatt gactgctcag 1020
 tagctatgaa ggtgactgt actatgaaac cagtgcctac tgcttaggca atcggcccca 1080
 ctgcttacia tgaattgtac agttacgttt tctggtacat agacttgatc acaccagaag 1140
 tataatccat cttaaaaatg gaaaaaata gagaatattt tgcgatgagc atctatgcag 1200
 atgattcttg attttgettc ttttggccct ttctaagtta tgcccttget gctatatgtc 1260
 acatctacgc ttttactgaa catactcttc ttatgtactc ggaaactggt agtccaaact 1320

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 tcataaaagt tgcagtaac 1339

<210> SEQ ID NO 7
 <211> LENGTH: 1371
 <212> TYPE: DNA
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 7

ccttgatct ccgtggaagc agataaagtt gcaaaaagag ctgtggttgt tggctgtggc 60
 ggcaggttcc cggtcgaaaa ggacgccaaa gaggaagtaa agctcttctt gggtaatgct 120
 ggaactgcaa tgcggccatt gacggcagct gtagttgctg ctggtgaaa tgcaacgtat 180
 gttttcttta tcctagttaa ataagtatga gatccatggg tatgtttga gactgatcat 240
 gtcytttatt taaaaaaaa acttcagtta tgtgcttgat ggagtaccaa gaatgagga 300
 gcgacctatt ggtgacttgg ttgtcggttt gcaacaactc ggcgcaratg ctgattgttt 360
 ccttggcact aactgccac ctgtccgtat caatggcaaa ggagggctac ctggtggcaa 420
 ggtagctac tcgtgaaatt gcatggtatg tacttttgag cacattcagt tctctgtcaa 480
 agaaaacaaa tattatattg gtagtacgat caatgagacc ttacacaatg cactaaagtt 540
 gaagaaccaa taracattga ataatttcaa tacttgagta ttgcatgatc aatcatagat 600
 tgacacttaa tattgaataa tttcacaggt taagctctct ggttcatta gcagtcaata 660
 cctgagttcc ttgctgatgg ctgctccttt ggctcttgaa gatgtcgaga ttgaaatcat 720
 tgataaactg atctccgttc cttacgttga aatgacattg aaattgatgg agcgttttgg 780
 cgtgactgcg gcgattctg atagttggga cagattcwac atcaaggag gacaaaaata 840
 caagtaagtt ctacattgct ttacttcttc tgatagtgga gtacaaaagc atgattctaa 900
 ttttctgtct acccacatat ttatagacgc accccattt gaattattga taaactagga 960
 aataaatatt gtacaaaatc tgtcgactgc tcagtagctc tgaaggcgca ctgtgctagg 1020
 aaaccagtgt ctactgccta ggcaatcaac cttctacct acaatgcatt gcacagttct 1080
 gttttctgga catatacttg atcacaccag aagtatcatc catcttaaaa ttggactgar 1140
 aatagagaac atcggcagtt gtttggcaat gagcatctat gcagatgatt cttgattttg 1200
 cttcttttgg cccttttcta tcttatgccc ttgctgcoat atggccatc aggatacaca 1260
 tccatgcttt tactgaacat actcttatgt actcggaaac tgtagttta aacttcataa 1320
 aagttgcagt aaccatttcc taaacgagcc atctgttctc ggaatcgaca g 1371

<210> SEQ ID NO 8
 <211> LENGTH: 1280
 <212> TYPE: DNA
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 8

acgcaaaga ggaagtaaag ctcttcttgg gtaatgctgg aactgcaatg cgtccattga 60
 cggcagctgt agtagctgct ggtggaaatg caacgtatgt tttctttatc ctagttgaaa 120
 taagtatgag atccatgggt atgtttggag actgatcgtg tcttttatta aaaaaaaca 180
 cttcagttat gtgcttgatg gagtaccaag aatgagggag cgacctattg gtgacttagt 240
 tgtaggtttg caacaactcg ggcagatgc tgattgtttc cttggcacta actgcccacc 300
 tgttcgtatc aatggcaaag gagggctacc tgggtggcaag gttagctact tgtgaacttg 360
 catgttatgt acttttgtgc acattcagtt ctctgtcaaa gaaaaataat attattatgg 420
 gtagtacgat gcaacgagc cttacaccgt gcactaaagt ttaagaacca ataacattg 480

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aataaattca atacttgagt attgctgat caatcataga ttggcgctta acattgaata 540
atttcacagg ttaagctctc tggttccatt agcagtcaat acctgagttc cttgctgatg 600
gctgctcctt tggctcttga ggatgctgag attgaaatca ttgataaact gatctccggt 660
ccttacgttg aaatgacatt gaaattgatg gacggttttg gcgtgactgc ggagcattct 720
gatagttggg acagattcta cattaagggg ggacaaaagt acaagtaagt tctacattgc 780
tttacttctt ctgatagtgg agtacaaaag catgattcta gttttcagtc taccxaaata 840
tttatagacg caccgccatt tgaattattg ataaactagg aaataaatat tgtacaaaat 900
ctgtcgactg ctgtagctt tcaagggcgc actgtactag gaaaccagtg cctactgcct 960
aggcaatcga cctgctacc tacaatgcat tgcacagttc tgttttctgg acatagactt 1020
gatcacacca gaagtatcat ccatcttaa attggactga aaatagagga tatcggcagt 1080
tgtttgcaa tgagcatcta tgcagatgat acttgatttt gcttcttttg acccttttta 1140
tcttaggcc ttgctgcat atggccatc aggatcacaca tccatgctta tactgaacat 1200
actcttatgt actcggaac tgtagttca aactcataa aagttgcagt aaccatttcc 1260
taaacgagcc atccgttctc 1280

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<210> SEQ ID NO 9
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Triticum aestivum

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<400> SEQUENCE: 9

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Met Ala Met Ala Ala Ala Ala Thr Val Ala Ala Ser Ala Ser Ser Ser
1           5           10           15
Ala Val Ser Leu Asp Arg Ala Ala Pro Ala His Pro Arg Arg Leu Arg
20          25          30
Met Pro Ala Ala Arg Ala Ala His Arg Gly Ala Val Arg Leu Trp Gly
35          40          45
Pro Arg Gly Ala Ala Ala Arg Ala Thr Ser Val Ala Ala Pro Ala Ala
50          55          60
Pro Ala Gly Ala Glu Glu Val Val Leu Gln Pro Ile Arg Glu Ile Ser
65          70          75          80
Gly Ala Val Gln Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu
85          90          95
Leu Leu Ser Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu
100         105         110
Asn Ser Glu Asp Val His Tyr Met Leu Glu Ala Leu Glu Ala Leu Gly
115         120         125
Leu Ser Val Glu Ala Asp Lys Val Ala Lys Arg Ala Val Val Val Gly
130         135         140
Cys Gly Gly Arg Phe Pro Val Glu Lys Asp Ala Lys Glu Glu Val Lys
145         150         155         160
Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala
165         170         175
Val Val Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro
180         185         190
Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Gln Gln
195         200         205
Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr Asn Cys Pro Pro Val
210         215         220

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Arg Ile Asn Gly Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser
 225 230 235 240
 Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ser Leu Leu Met Ala Ala Pro
 245 250 255
 Leu Ala Leu Glu Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser
 260 265 270
 Val Pro Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val
 275 280 285
 Thr Ala Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly
 290 295 300
 Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr Val Glu Gly Asp Ala Ser
 305 310 315 320
 Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val
 325 330 335
 Thr Val Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe
 340 345 350
 Ala Glu Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Asp Thr
 355 360 365
 Ser Val Thr Val Thr Gly Pro Pro Arg Gln Pro Phe Gly Arg Lys His
 370 375 380
 Leu Lys Ala Val Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met
 385 390 395 400
 Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg
 405 410 415
 Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Val Ala Ile
 420 425 430
 Arg Thr Glu Leu Thr Lys Leu Gly Ala Thr Val Glu Glu Gly Pro Asp
 435 440 445
 Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn Ile Thr Ala Ile Asp
 450 455 460
 Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys
 465 470 475 480
 Ala Glu Val Pro Val Thr Ile Arg Asp Pro Gly Cys Thr Arg Lys Thr
 485 490 495
 Phe Pro Asn Tyr Phe Asp Val Leu Ser Thr Phe Val Lys Asn
 500 505 510

<210> SEQ ID NO 10
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

<400> SEQUENCE: 10

acagtgagga tgtccactac atgcttga

28

<210> SEQ ID NO 11
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

<400> SEQUENCE: 11

acttctctga cagagaacag aagtgtgcac

30

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<210> SEQ ID NO 12
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 12

 ttgtgtaagg tcgcattgat cgtactacca 30

<210> SEQ ID NO 13
 <211> LENGTH: 34
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 13

 gaaaactaga atcatgcttt tgtactccac tatc 34

<210> SEQ ID NO 14
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 14

 acgccaaaga ggaagtaaag ct 22

<210> SEQ ID NO 15
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 15

 tccaaacata cccatggatc tcatact 27

<210> SEQ ID NO 16
 <211> LENGTH: 15
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 16

 cgcattgcag ttcca 15

<210> SEQ ID NO 17
 <211> LENGTH: 13
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

 <400> SEQUENCE: 17

 cattgcaatt cca 13

<210> SEQ ID NO 18
 <211> LENGTH: 22
 <212> TYPE: PRT
 <213> ORGANISM: Triticum aestivum

 <400> SEQUENCE: 18

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Phe Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val
 1 5 10 15
 Val Ala Ala Gly Gly Asn
 20

 <210> SEQ ID NO 19
 <211> LENGTH: 510
 <212> TYPE: PRT
 <213> ORGANISM: Triticum aestivum

 <400> SEQUENCE: 19

 Met Ala Met Ala Ala Ala Ala Thr Val Ala Ala Ser Ala Ser Ser Ser
 1 5 10 15
 Ala Val Ser Leu Asp Arg Ala Ala Pro Ala His Pro Arg Arg Leu Arg
 20 25 30
 Met Pro Ala Ala Arg Ala Ala His Arg Gly Ala Val Arg Leu Trp Gly
 35 40 45
 Pro Arg Gly Ala Ala Ala Arg Ala Thr Ser Val Ala Ala Pro Ala Ala
 50 55 60
 Pro Ala Gly Ala Glu Glu Val Val Leu Gln Pro Ile Arg Glu Ile Ser
 65 70 75 80
 Gly Ala Val Gln Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu
 85 90 95
 Leu Leu Ser Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu
 100 105 110
 Asn Ser Glu Asp Val His Tyr Met Leu Glu Ala Leu Glu Ala Leu Gly
 115 120 125
 Leu Ser Val Glu Ala Asp Lys Val Ala Lys Arg Ala Val Val Val Gly
 130 135 140
 Cys Gly Gly Arg Phe Pro Val Glu Lys Asp Ala Lys Glu Glu Val Lys
 145 150 155 160
 Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala
 165 170 175
 Val Val Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro
 180 185 190
 Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Gln Gln
 195 200 205
 Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr Asn Cys Pro Pro Val
 210 215 220
 Arg Ile Asn Gly Lys Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser
 225 230 235 240
 Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ser Leu Leu Met Ala Ala Pro
 245 250 255
 Leu Ala Leu Glu Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser
 260 265 270
 Val Pro Tyr Val Glu Met Thr Leu Lys Leu Met Glu Arg Phe Gly Val
 275 280 285
 Thr Ala Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly
 290 295 300
 Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr Val Glu Gly Asp Ala Ser
 305 310 315 320
 Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val
 325 330 335
 Thr Val Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe
 340 345 350

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Ala Glu Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Asp Thr
 355 360 365

Ser Val Thr Val Thr Gly Pro Pro Arg Gln Pro Phe Gly Arg Lys His
 370 375 380

Leu Lys Ala Val Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met
 385 390 395 400

Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg
 405 410 415

Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Val Ala Ile
 420 425 430

Arg Thr Glu Leu Thr Lys Leu Gly Ala Thr Val Glu Glu Gly Pro Asp
 435 440 445

Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn Ile Thr Ala Ile Asp
 450 455 460

Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys
 465 470 475 480

Ala Glu Val Pro Val Thr Ile Arg Asp Pro Gly Cys Thr Arg Lys Thr
 485 490 495

Phe Pro Asn Tyr Phe Asp Val Leu Ser Thr Phe Val Lys Asn
 500 505 510

<210> SEQ ID NO 20

<211> LENGTH: 515

<212> TYPE: PRT

<213> ORGANISM: *Oryza sativa*

<400> SEQUENCE: 20

Met Ala Ala Thr Met Ala Ser Asn Ala Ala Ala Ala Ala Ala Val Ser
 1 5 10 15

Leu Asp Gln Ala Val Ala Ala Ser Ala Ala Phe Ser Ser Arg Lys Gln
 20 25 30

Leu Arg Leu Pro Ala Ala Ala Arg Gly Gly Met Arg Val Arg Val Arg
 35 40 45

Ala Arg Gly Arg Arg Glu Ala Val Val Val Ala Ser Ala Ser Ser Ser
 50 55 60

Ser Val Ala Ala Pro Ala Ala Lys Ala Glu Glu Ile Val Leu Gln Pro
 65 70 75 80

Ile Arg Glu Ile Ser Gly Ala Val Gln Leu Pro Gly Ser Lys Ser Leu
 85 90 95

Ser Asn Arg Ile Leu Leu Leu Ser Ala Leu Ser Glu Gly Thr Thr Val
 100 105 110

Val Asp Asn Leu Leu Asn Ser Glu Asp Val His Tyr Met Leu Glu Ala
 115 120 125

Leu Lys Ala Leu Gly Leu Ser Val Glu Ala Asp Lys Val Ala Lys Arg
 130 135 140

Ala Val Val Val Gly Cys Gly Gly Lys Phe Pro Val Glu Lys Asp Ala
 145 150 155 160

Lys Glu Glu Val Gln Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg
 165 170 175

Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly Asn Ala Thr Tyr Val
 180 185 190

Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val
 195 200 205

Val Gly Leu Lys Gln Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr

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210	215	220
Glu Cys Pro Pro Val Arg Val Lys Gly Ile Gly Gly Leu Pro Gly Gly 225 230 235 240		
Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ala Leu 245 250 255		
Leu Met Ala Ala Pro Leu Ala Leu Gly Asp Val Glu Ile Glu Ile Ile 260 265 270		
Asp Lys Leu Ile Ser Ile Pro Tyr Val Glu Met Thr Leu Arg Leu Met 275 280 285		
Glu Arg Phe Gly Val Lys Ala Glu His Ser Asp Ser Trp Asp Arg Phe 290 295 300		
Tyr Ile Lys Gly Gly Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr Val 305 310 315 320		
Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile 325 330 335		
Thr Gly Gly Thr Val Thr Val Gln Gly Cys Gly Thr Thr Ser Leu Gln 340 345 350		
Gly Asp Val Lys Phe Ala Glu Val Leu Glu Met Met Gly Ala Lys Val 355 360 365		
Thr Trp Thr Asp Thr Ser Val Thr Val Thr Gly Pro Pro Arg Glu Pro 370 375 380		
Tyr Gly Lys Lys His Leu Lys Ala Val Asp Val Asn Met Asn Lys Met 385 390 395 400		
Pro Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly 405 410 415		
Pro Thr Ala Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu 420 425 430		
Arg Met Val Ala Ile Arg Thr Glu Leu Thr Lys Leu Gly Ala Ser Val 435 440 445		
Glu Glu Gly Pro Asp Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn 450 455 460		
Ile Thr Ala Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe 465 470 475 480		
Ser Leu Ala Ala Cys Ala Asp Val Pro Val Thr Ile Arg Asp Pro Gly 485 490 495		
Cys Thr Arg Lys Thr Phe Pro Asn Tyr Phe Asp Val Leu Ser Thr Phe 500 505 510		
Val Arg Asn 515		

<210> SEQ ID NO 21
 <211> LENGTH: 514
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Consensus sequence
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(4)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(8)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (10)..(11)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (344)..(344)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (383)..(383)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (486)..(486)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 21

Xaa Xaa Xaa Xaa Met Ala Xaa Xaa Ala Xaa Xaa Ala Ala Xaa Xaa Ser
1          5          10          15

Leu Asp Xaa Xaa Ser Xaa Xaa Arg Ala Ala Phe Xaa His Arg Arg Arg
20          25          30

Leu Arg Xaa Pro Ala Ala Xaa Arg Xaa Xaa His Arg Gly Xaa Val Arg
35          40          45

Xaa Arg Gly Xaa Arg Glu Ala Xaa Xaa Xaa Ala Xaa Ala Xaa Ser Ser
50          55          60

Xaa Xaa Xaa Ala Xaa Xaa Ala Gly Ala Glu Glu Xaa Val Leu Gln Pro
65          70          75          80

Ile Arg Glu Ile Ser Gly Ala Val Gln Leu Pro Gly Ser Lys Ser Leu
85          90          95

Ser Asn Arg Ile Leu Leu Leu Ser Ala Leu Ser Glu Gly Thr Thr Val
100         105         110

Val Asp Asn Leu Leu Asn Ser Glu Asp Val His Tyr Met Leu Glu Ala
115         120         125

Leu Glu Ala Leu Gly Leu Ser Val Glu Ala Asp Lys Val Ala Lys Arg
130         135         140

Ala Val Val Val Gly Cys Gly Gly Arg Phe Pro Val Glu Lys Asp Ala
145         150         155         160

Lys Glu Glu Val Gln Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg
165         170         175

Pro Leu Thr Ala Ala Val Thr Ala Ala Gly Gly Asn Ala Thr Tyr Val
180         185         190

Leu Asp Gly Val Pro Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val
195         200         205

Val Gly Leu Gln Gln Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr
210         215         220

Xaa Cys Pro Pro Val Arg Xaa Xaa Gly Ile Gly Gly Leu Pro Gly Gly
225         230         235         240

Lys Val Lys Leu Ser Gly Ser Ile Ser Ser Gln Tyr Leu Ser Xaa Leu
245         250         255

Leu Met Ala Ala Pro Leu Ala Leu Glu Asp Val Glu Ile Glu Ile Ile
260         265         270

Asp Lys Leu Ile Ser Xaa Pro Tyr Val Glu Met Thr Leu Arg Leu Met
275         280         285

Glu Arg Phe Gly Val Lys Ala Glu His Ser Asp Ser Trp Asp Arg Phe
290         295         300

Tyr Ile Lys Gly Gly Gln Lys Tyr Lys Ser Pro Gly Asn Ala Tyr Val
305         310         315         320

Glu Gly Asp Ala Ser Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile
325         330         335

Thr Gly Gly Thr Val Thr Val Xaa Gly Cys Gly Thr Thr Ser Leu Gln

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340	345	350
Gly Asp Val Lys Phe Ala Glu Val Leu Glu Met Met Gly Ala Lys Val 355 360 365		
Thr Trp Thr Asp Thr Ser Val Thr Val Thr Gly Pro Pro Arg Xaa Pro 370 375 380		
Gly Arg Lys His Leu Lys Ala Val Asp Val Asn Met Asn Lys Met Pro 385 390 395 400		
Asp Val Ala Met Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly Pro 405 410 415		
Thr Ala Ile Arg Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu Arg 420 425 430		
Met Val Ala Ile Arg Thr Glu Leu Thr Lys Leu Gly Ala Ser Val Glu 435 440 445		
Glu Gly Pro Asp Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn Ile 450 455 460		
Thr Ala Ile Asp Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe Ser 465 470 475 480		
Leu Ala Ala Cys Ala Xaa Val Pro Val Thr Ile Arg Asp Pro Gly Cys 485 490 495		
Thr Arg Lys Thr Phe Pro Asn Tyr Phe Asp Val Leu Ser Thr Phe Val 500 505 510		
Arg Asn		

What is claimed is:

1. A wheat plant comprising:
a human-induced, non-transgenic mutation in an EPSPS gene in each of the wheat plant's A and D genomes, wherein the EPSPS gene of the A genome is homozygous for at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the D genome is homozygous for at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the mutations in the EPSPS genes contribute to a wheat plant having resistance to glyphosate as compared to a wild type wheat plant.
2. The wheat plant of claim 1 further comprising:
a human-induced, non-transgenic mutation in the EPSPS gene of the wheat plant's B genome.
3. The wheat plant of claim 2, wherein the human-induced, non-transgenic mutation in the EPSPS gene of the B genome encodes an EPSPS polypeptide having a mutation recited in Table 4.
4. The wheat plant of claim 1, wherein the wheat is *Triticum aestivum*.
5. A wheat seed, plant part, or progeny thereof from the wheat plant of claim 1.
6. Wheat grain from the wheat plant of claim 1.
7. Flour comprising the wheat grain of claim 6.
8. A food product comprising a component of the wheat plant of claim 1.
9. A food product comprising the flour of claim 7.
10. A wheat plant comprising:
a human-induced, non-transgenic mutation in an EPSPS gene in each of the wheat plant's A and B genomes, wherein the EPSPS gene of the A genome is homozygous for at least two mutations and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) and a proline to serine change at amino acid position 172 (P172S) of SEQ ID NO. 9, and wherein the EPSPS gene of the B genome is homozygous for at least one mutation and encodes an EPSPS polypeptide comprising a threonine to isoleucine change at amino acid position 168 (T168I) of SEQ ID NO. 9, and wherein the mutations in the EPSPS genes contribute to a wheat plant having resistance to glyphosate as compared to a wild type wheat plant.
11. The wheat plant of claim 10 further comprising:
a human-induced, non-transgenic mutation in the EPSPS gene of the wheat plant's D genome.
12. The wheat plant of claim 11, wherein the human-induced, non-transgenic mutation in the EPSPS gene of the D genome encodes an EPSPS polypeptide having a mutation recited in Table 4.
13. The wheat plant of claim 10, wherein the wheat plant is *Triticum aestivum*.
14. The wheat plant of claim 10, wherein the wheat plant is *Triticum turgidum ssp durum*.
15. A wheat seed, plant part, or progeny thereof from the wheat plant of claim 10.
16. Wheat grain from the wheat plant of claim 10.
17. Flour comprising the wheat grain of claim 16.
18. A food product comprising a component of the wheat plant of claim 10.
19. A food product comprising the flour of claim 17.

* * * * *